10

Quality control in yoghurt manufacture

10.1 Introduction

The quality of any food product can be defined against a wide range of criteria, including, for example the chemical, physical, microbiological and nutritional characteristics, or simply in relation to its overall appeal to potential consumers. As a result, quality has to be judged by a range of tests with varying degrees of objectivity, and yet all of them can be useful in ensuring that a product:

- is safe for human consumption with respect to both chemical or microbial contamination;
- conforms to any regulations enshrined in law, or advisory/statutory requirements laid down by public health or other local authorities/agencies;
- is capable of achieving a specified shelf life without spoilage;
- has as high an organoleptic standard as can be achieved within the existing constraints of manufacture or marketing.

An examination of some of these points implies, naturally enough, a critical laboratory assessment of the retail product, but it is essential to bear in mind that the end product can only be as sound as the raw materials from which it is made and, in hygienic terms, as "clean" as the plant in which it was manufactured. This breadth of potential for conflict means that quality control must be regarded as an all embracing concept and, furthermore, one that demands constant attention. Thus, enthusiasm in response to a crisis is of little value in maintaining standards and the successful companies are those that rate quality appraisal as a high priority. Even small firms with minimal facilities can achieve a great deal by maintaining records of simple features like incubation times, product acidity and so on, and even though the services of a consultant may be required for more specialised examinations, the value of routine monitoring should never be underestimated.

Indeed, routine has become the lynchpin of successful manufacture and is enshrined in two compatible and, to some extent, overlapping concepts – good manufacturing practice (GMP) and the hazard appraisal (analysis) critical control points

(HACCP) system. The starting point has to be the current legislative controls in the country in question and, in England, Scotland and Wales, for example, a dairy product has to conform to the following:

- Food Safety Act (Anon., 1990)
- Dairy Products (Hygiene) Regulations (SI, 1995a)
- Dairy Products (Hygiene) (Scotland) Regulations (SI, 1995b)
- Miscellaneous Food Additives Regulations (SI, 1995c)
- Sweeteners in Foods Regulations (SI, 1995d)
- Colours in Foods Regulations (SI, 1995e)
- Food Labelling Regulations (SI, 1996)
- Weights and Measures Act (Anon., 1985)
- Weights and Measures Regulations (SI, 1987)

Specifically for yoghurt, there are codes of practice that may or not be observed according to views of the producer (MAFF, 1975; DTF, 1983). In all European Union (EU) countries, labelling is covered by Council Directive 79/112 (EU, 1979) and most producing regions will have similar patterns of legislation (Pappas, 1988; Anon., 1989; Glaeser, 1992).

Assuming that, in theory at least, neither the product nor the packaging contravenes any of these Regulations, then the manufacturer must be able to demonstrate that compliance with the Regulations is being achieved in actual practice. The key word is, of course, demonstrate, for while it is anticipated that any manufacturer can produce a faulty batch of produce, what the same manufacturer must be able to show is that the fault arose despite due diligence being shown by all concerned. It was this blanket responsibility that gave rise to the HACCP concept, and the basic principles of the system are now widely accepted as the basis for responsible operation of a factory.

10.2 Principles of HACCP

10.2.1 Brief introduction

In theory, the only way of ensuring that every carton of yoghurt from a given production line is safe, from a chemical or microbiological standpoint, is to test every carton! Clearly, such a suggestion is totally ludicrous, so that instead, a representative group of cartons is withdrawn against a sampling plan appropriate for the product and the history of the plant. However, whilst this approach is essential to confirm that preset standards of hygiene are being met and that potential contaminants are at a low level or absent, the procedure can never prevent some spoiled cartons from reaching the consumer. Consequently, the emphasis within quality assurance has turned to the avoidance of problems, a concept that forms the basis of HACCP. The HACCP system aims to identify specific hazards that, if they arose, could adversely affect the safety of a food and to put in place a procedure that will either prevent a hazard arising or will be able to control the situation in a manner that reduces the risk to the consumer (Vazquez, 1988; Pierson and Corlett, 1992; Corlett, 1992; WHO, 1993; Asperger, 1994; Mortimore and Wallace, 1994; IDF, 1994a; van Schothorst and Kleiss, 1994; Loken, 1995; FAO, 1995; Anon., 1997a, 1998a).

In particular, the system identifies seven aspects of production that merit constant attention and these aspects are enshrined in seven principles:

- First – any potential hazards associated with yoghurt production from the growth/collection of raw materials through to manufacture and distribution must be identified and an assessment made of: (a) the likelihood that a given hazard will arise, and (b) the preventative measures that are necessary to reduce any inherent risks.
- Second – the precise points in the above sequence that can be controlled in order to eliminate a hazard or minimise the risk of occurrence must also be identified. If failure to control a particular hazard is a risk to public health, then the step in the process is regarded as a critical control point (CCP); if no major risk is involved, the step may be identified as a control point (CP). For example, the filling machine is a CCP, because contamination with a pathogen could present a direct risk to the consumer, whereas the failure to empty a waste bin in the same area could be treated as a CP because, however undesirable with respect to the growth of potential spoilage organisms, the failure is not likely to result in a consumer health problem. Similarly, it is important that a manufacture has control over the chemical composition of a yoghurt and the details on the label, but again such points need only be graded as CPs.
- Third – there must an established set of targets which must be achieved in order for a Section to claim control over a CCP/CP, e.g. total colony counts on product contact surfaces (CCP) or the viscosity of stirred yoghurt with agreed tolerances (CP).
- Fourth – a monitoring system must be established to record that particular facets of production are under control.
- Fifth – if the monitoring procedure indicates that a CCP/CP is not under control, then an agreed programme of corrective action must be capable of immediate implementation.
- Sixth – there must be procedures for verification that the HACCP system is working throughout the factory, e.g. the introduction of supplementary checks to ensure that the principal components of the system are operating to the required standard.
- Seventh – a system of documentation must be in place that records accurately the details of all operations, e.g. times/temperatures and microbiological parameters, but also the responsibilities of the individual operators associated with that specific section of the process.

At first glance, this approach may appear daunting but, if each stage in a manufacturing process is identified and considered as a separate entity, then isolating the areas of risk can bring considerable benefits to a manufacturer. For example, retailers have confidence in a company that has proper control over its manufacturing procedures and, for this reason, the introduction of HACCP is fast becoming an essential of operation in the commercial world. It is important, however, that no two production plants are ever identical, and hence the personnel responsible for routine examinations must exercise their discretion as to which tests are both desirable and feasible in a given situation (see also Cullor, 1997; Gardner, 1997).

Although the systems employed to monitor the quality of yoghurt fall within the HACCP umbrella, each aspect of production has, by its very nature, to be assessed

in a different way, and hence it is appropriate to deal with the separate facets of quality on an individual basis. It is relevant in this context that, although quality control is a broad concept, hygiene is inevitably a dominant feature and excellent accounts of the principles and practice of microbiological quality control in the dairy industry have been published by Lück and Gavron (1990), Jervis (1992) and IDF (1992e); anyone likely to be concerned with the hygienic aspects of production would be well advised to consult these works.

10.2.2 Implementation of a HACCP system

The successful implementation of a HACCP system demands, perhaps above all, the whole-hearted commitment of top management and the willingness of that same management to support those charged with running the monitoring procedures on a day-to-day basis. In return, each operative must know exactly the nature and extent of his/her responsibilities and that any decisions made in the interests of the Company within the confines of that remit will be approved irrespective of any adverse financial implications. To build up the necessary personnel structure and confidence to ensure smooth operation is not an easy task but, once the essential framework is in place and functional, the anticipated freedom from unforeseen crises is reward enough for the effort.

The first stage is the easiest and involves little more than the production/quality control managers drafting a flow-diagram of the overall process and annotating it with indications of the likely control points. A typical example for set natural yoghurt is shown in Fig. 10.1 (Kalantzi, personal communication) and the relative importance of the identified CPs will need to be assessed. For example, both the heat treatment and inoculation steps might be considered as critical (i.e. CCP), for if the vegetative cells of pathogens survive the heating stage and starter activity is poor, a serious public health risk could arise. By contrast, dusty cartons could lead to an avalanche of product returns as moulds grow on the surface of the yoghurt, but the actual risk of illness for any given consumer would be negligible and constitutes a CP.

Once the overall scenario has been agreed, further details have to be added. Table 10.1 gives an example of the type of reception tests that might be applied to the raw milk arriving from a farm or collection centre (Kalantzi, personal communication). Some typical specifications for these attributes are given later (see Section 10.4.1) and the selection of tests to be completed may have to be adjusted according to the situation in the laboratory. For example, the measurement of pH may be sufficient for routine purposes, provided that calibration of the meter is carried out regularly, so that the measure of acidity or clot on boiling test might be omitted. However, the total colony count might be applied on a regular basis at least once weekly to gain a more accurate picture of microbial quality. Details of targets and tolerances will be a matter for local negotiation, but all manufacturers should be seeking zero tolerance for inhibitory substances, that is, below the level of detection by the best procedure available in the country concerned. Thus, not only can antibiotic residues lead to partial starter failure, but the passage of β-lactam antibiotics like penicillin into the food chain can cause allergic reactions and even death amongst susceptible consumers. For this latter reason alone, the reception of raw milk could be rated as a CCP.

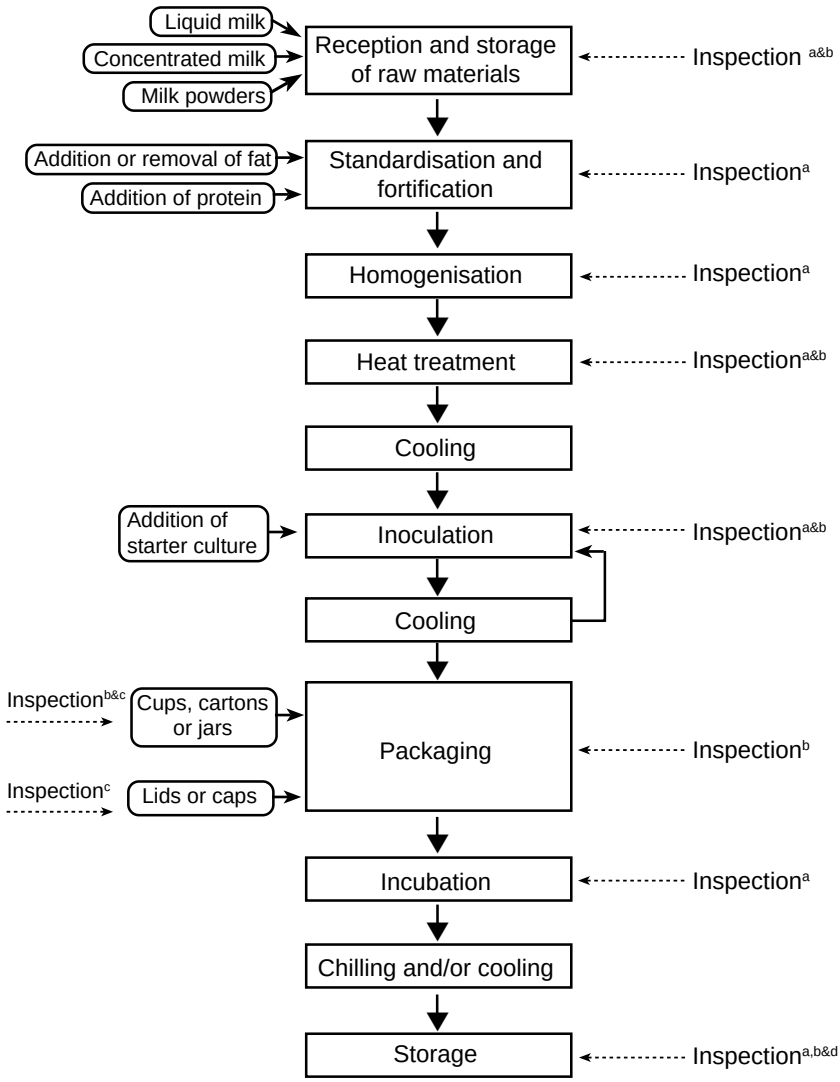


Fig. 10.1 Typical HACCP scheme for the production of set natural yoghurt
a, Physicochemical; b, microbiological; c, visual; d, organoleptic.

A similar chart can be drawn-up for other raw materials, for example, milk powder or fruit, or for a partly processed product. A case in point might be the yoghurt base prior to the addition of fruit for, if the retail product is to be acceptable to consumers, this yoghurt base must have certain defined properties with respect to acidity and viscosity; if the base is suspect, there may be little point in wasting large volumes of expensive fruit. However, these simple records are specific requirements that help to underpin the overall system, a point that is highlighted by the small section of interaction chart shown in Fig. 10.2 (Kalantzi, personal communication).

Table 10.1 Example worksheet for recording the quality of raw milk at reception

Description of the Product: Raw Milk

Type: Cow's, goat's, sheep's or buffalo's milk
Combination of Milks: % in final product (optional or if applicable)
Characteristics: see below

Analysis	Target	Tolerance	Reject
Physicochemical			
Moisture, Fat, Protein } ($g\,100\,g^{-1}$)			
Titratable acidity (%)			
pH			
Specific gravity			
Temperature (°C)			
Antibiotics			
Clot on boiling			
Microbiological (cfu ml^{-1})			
Total viable count, Thermoduric count, Psychrotrophic count } Optional			
Organoleptic			
Colour			
Odour			
Foreign objects			
Origin:			
Duration at and temperature: Farm and/or collection centers:			
During transport:			
In silos:			
On-site storage temperature: <5°C			

Thus, assuming that the yoghurt base is moving along the central axis, the chart shows just some of the questions that need to be answered as the HACCP scheme evolves. In some cases, it may be agreed that the existing operation is satisfactory and only the following will need to be written in the HACCP manual:

- The identified point in the process and the required standards, e.g. the times and temperatures that must be achieved during heat treatment of the milk;
- The importance of loss of control with respect to the process, i.e. is it a CP or CCP and what are the implications of failure?;
- The designation of operatives/supervisors for each operation, and the procedures for reporting; and
- Corrective actions that may be necessary, with clear statements of responsibilities and expected outcomes.

However, at other points, new responsibilities may emerge and actions or procedures which were once taken for granted will have to be formalised in relation to questions, such as how often should samples be taken or instruments checked?; who should carry out the work?; what checks are essential?; and why, and to whom should the results be shown for analysis/action?

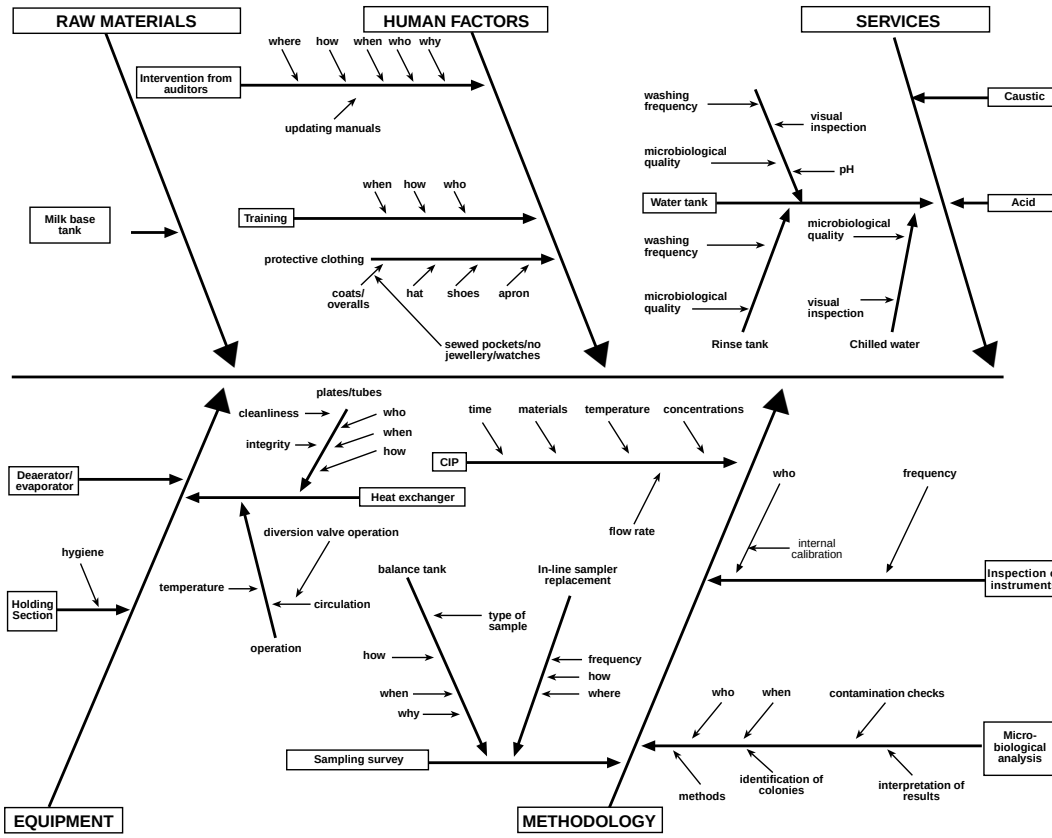


Fig. 10.2 Illustration of an interaction chart that highlights records that are required in a yoghurt factory

In addition, a protocol must be developed to audit the performance of the system, for whether the auditors are internal or from outside the company, regular inspections of the operation of the agreed HACCP procedures are essential.

Obviously, the initial establishment of a HACCP system will be both time consuming and demanding on the patience of the personnel involved, but most companies agree that the benefits more than compensate for the tedium of implementation. Whether or not HACCP should form part of a total quality management package is a matter for debate and the advantages and disadvantages of introducing systems like ISO 9000 are best debated within individual companies (see also BSI, 1991a, 1993, 1994; Lamprecht, 1993; Bolton, 1997).

10.3 Monitoring of process plant

The acidity of yoghurt means that spoilage is often associated with yeasts and moulds and the latter in particular often have their origin in the microbial flora of the air. The control of the atmosphere within the factory environment will depend on the level of air cleanliness that is essential for completion of a particular

operation (Bruderer and Schicht, 1987; Schicht, 1989, 1991; Fitzpatrick, 1990; Blümke, 1993). For example, laminar flow cabinets may be able to provide a local, high quality region for certain manual mixing operations (Audidier, 1996) and high efficiency particulate air (HEPA) filtration systems can reduce the overall microbial loading in the air by 90% (Hampson and Kaiser, 1995). It is important, however, that plant designed to induce air flow through a filling room or production area can also act as a source of contamination (Anon., 1988b) and some specifications for air quality have been published by the US Federal Standards – 209D (Anon., 1988c). Packaging materials stored adjacent to the filling line can also cause problems, as can the unnecessary movement of personnel and these aspects of plant operation deserve constant attention. If the problem of airborne contamination becomes really serious, then one of the air sampling methods described by the United States Public Health Service (USPHS, 1959) Ottaviani and Franceschetti (1983), Pfleger (1985) and APHA (American Public Health Association) (1992) could be employed to isolate the source(s) of the invading propagules.

Although yeasts and moulds of atmospheric origin can be important, especially at certain times of the year (Gregory, 1961), it is the contact surfaces of the plant that usually pose the greatest threat to product security. In small factories, strict attention to hygiene and visual inspections may be supplemented by a bioluminescence test for total adenosine-5-triphosphate (ATP). In this test, a small area of plant surface (perhaps 100 cm^2) is carefully swabbed and any biological material collected (i.e. food and microbial contaminants) is transferred to a solution containing firefly luciferase and reduced luciferin (Anon., 1997a). In this situation, the ATP is reduced to adenosine monophosphate (AMP) and energy released is emitted as light. As the quantity of light recorded by a photometer is proportional to the initial level of ATP, the photometer reading will give an indication of the total level of biological material in the reaction fluid. If the swabbing procedure has been carried out correctly, then the photometer reading is, in effect, a measure of the state of hygiene of the plant surface (Pettipher, 1993). Obviously the readings are not intended to correlate with a microbial count, but there is an excellent correlation between clean surfaces and low levels of ATP. In large factories, the same approach can be used for regular monitoring of tanks, pipelines and other equipment, but it is often supplemented by specific tests for the general microflora and/or specific organisms.

However, whatever tests are employed, it is essential for the maintenance of hygienic conditions that they are applied routinely, for individual readings are in themselves meaningless; only when values for a typical, high standard of hygiene have been established for a given plant, along with acceptable tolerances, do the results of any microbiological/hygiene test become valuable.

For large items of equipment, one technique of almost universal application is the swab method (Harrigan and McCance, 1976; BSI, 1991b; APHA, 1992; IDF, 1993a, 1996a), in which a damp swab of cotton gauze (or some approved alternative) is rubbed over a designated area of the contact surface. The swab is then agitated in a known volume of a physiologically neutral solution and once the microorganisms are deemed to have been removed from the swab, samples of the solution, diluted if necessary, are examined by the plate count method (BSI, 1984; see also IDF, 1989). Milk agar is a most useful medium for dairy equipment and after incubation at 30°C for 72 hours, a colony count is obtained which can readily be

transformed into a figure for colony forming units (cfu) 100 cm^{-2} of equipment surface.

The regular examination of selected or critical components of the production system can provide a useful indication of any decline in standards of cleaning and the rinse method (Harrigan and McCance, 1976; BSI, 1991b; APHA, 1992; IDF, 1993a, 1996a) can provide similar information for small items or containers. The performance of tests of this type on successive occasions (same operator and same conditions) is somewhat variable, hence the need for agreed tolerances, but it is trends away from the norm for any specific piece of equipment that are important. Some suggested standards for plant in contact with products prior to pasteurisation/heat treatment have been cited by Harrigan and McCance (1976):

cfu 100 cm^{-2}	Conclusion
500 (coliforms < 10)	Satisfactory
500–2500	Dubious
>2500 (coliforms > 100)	Unsatisfactory

With improved cleaning regimes, a total colony count of 200 cfu 100 cm^{-2} would be expected nowadays, and below 50 cfu 100 cm^{-2} for any plant containing pasteurised product (Lück and Gavron, 1990).

Different plants will achieve different levels of cleanliness even under ideal conditions and the manufacturer of yoghurt is perhaps fortunate that the product is fairly resistant to spoilage, at least of bacterial origin. Its reaction to yeasts and moulds is quite different, however, and if yeasts become the dominant contaminant, then numerous problems can be expected during retailing.

As an alternative to the procedures mentioned above, an agar contact method may be employed in which the sterile surface of a small Petri dish prefilled with an appropriate medium, or the exposed surface of an agar sausage (Cate, 1965), is placed in contact with the test surface. If the surface is not too heavily contaminated, then individual or clumps of micro-organisms adhere to the agar surface, and after incubation give rise to colonies that may be counted (Lück and Gavron, 1990). The results can again be related to a known area of plant surface, and as with data obtained in other ways, can provide an indication of the efficacy of the cleaning procedures.

It is clear, therefore, that examinations of this type are valuable as a means both of monitoring cleaning performance and of eliminating potential hazards, and the testing of raw materials has much the same function.

10.4 Examination of raw materials

10.4.1 Liquid milk

The basic ingredient of most yoghurt is whole milk or skimmed milk and hence the quality of the incoming milk is an important consideration. The methods of extracting representative samples will vary with the size of factory concerned, but it is essential that the portion examined truly reflects the quality of the bulk (IDF, 1990a, 1992a, 1995b).

The extent of any examination will depend on the scale of the operation, but may well include, as a minimum, some of the tests indicated in Table 10.2. If the milk is purchased in bulk, then the supplier can be expected to meet an agreed specification (see also Allen, 1995; SI, 1995b; Anon., 1994a; IDF, 1991d, 1995a, c, 1996b, d), and a typical set of figures might be:

Temperature on arrival	$<10°C$
Total colony count	$\leq 100\,000\,\text{cfu}\,\text{ml}^{-1}$ (target) ($<250\,000\,\text{cfu}\,\text{ml}^{-1}$ may well be acceptable in practice)
Inhibitory substances	$\leq 0.007\,\text{IU}\,\text{ml}^{-1}$ ($0.004\,\mu\text{g}\,\text{ml}^{-1}$)
Chemical composition	$\geq 3.0\,\text{g fat}\,100\,\text{g}^{-1}$
	$\geq 3.0\,\text{g protein}\,100\,\text{g}^{-1}$
Somatic cell count	$\leq 4.0\times 10^5\,\text{ml}^{-1}$
Freezing point depression	$\leq 0.520°C$
Titratable acidity	$\leq 0.2\%$ lactic acid

Table 10.2 Some tests that might be applied to raw whole or skimmed milk to be used in the production of yoghurt

Examination	Reason	Method	Reference
Total solids	Allows accurate concentration or fortification of process milk	Hydrometer	BSI (1959, 1962, 1973), IDF (1993c)
		Drying	IDF (1991a)
		Infrared	Andersen *et al.* (1993)
Fat	Legal or sensory requirements	Gerber	BSI (1989a), IDF (1981a, 1997a)
		Röse Gottlieb	IDF (1996e)
		Light scatter	Anon. (1987a), IDF (1990c)
		Infrared	Andersen *et al.* (1993) IDF (1996c)
Protein	Fortification requirement	Infrared	IDF (1996c)
		Dye-binding	IDF (1985)
		Kjeldahl	IDF (1993b)
Antibiotics	Prevents inhibition of starter culture	Delvotest® P[a]	IDF (1970, 1991b) Anon. (1994b)
Taints	Chemical taints can be detected in individual supplies of milk	Odour/"sniff" test	
Organochlorine	Pesticides can affect flavour or inhibit growth of starter cultures	Many	IDF (1991c)
Organophosphorus	As above	Chromatography	IDF (1990b)
Dirt	Straw, hair or soil	Filtration	BSI (1982)

[a] The alternative Lac-Tek® test is legally acceptable in some countries (see also IDF 1995a, 1997b; Zeng *et al.*, 1998).

While total colony counts are excellent for monitoring producer performance and, if required, making payment adjustments in-line with quality, the milk will have been processed long before the results of the count are known. In order to meet this criticism, a widely used alternative is the direct epifluorescent filter technique (DEFT), which gives a total viable count within 20 min (Sato *et al.*, 1986; Pettipher, 1993) or detection and enumeration of yeast in yoghurt (Rowe and McCann, 1990).

The availability of automated techniques means that the chemical composition of the incoming milk can be monitored as well (see later), but one essential test must be for inhibitory substances (IDF, 1986, 1991e, 1995a, 1997b). Thus, while minor variations in chemical composition may alter the quality of the end product and/or economics of the process, the presence of antibiotics in the milk can lead to total vat failure. The disc assay (IDF, 1970, 1991b; BSI, 1987) is able to detect 0.005 IU of penicillin G ml^{-1} of milk, while the more user friendly Delvotest® P (Anon., 1994b) can detect 0.004 IU of penicillin G ml^{-1} of milk in 2.5 hour; at a level of 0.006 IU of penicillin G ml^{-1} of milk, the Delvotest® is reported to be 100% accurate (Scannella *et al.*, 1997). More recently, the Lac-Tek® and Delvo-X-Press® βL-II tests have been introduced and these systems can identify a range of β-lactam antibiotics again at levels of 0.006 IU ml^{-1} of milk but, in this case, the detection time is around 7 min (Anon., 1997b). This rapid response means that all milk required for processing can be tested ahead of introduction into the production area. Alternatively, the Charm test(s) offers another alternative for checking for β-lactam residues (APHA, 1992), and standard methods are also cited for the high pressure liquid chromatography (HPLC) detection of sulphamethazine, the brilliant black reduction test for inhibitory substances, as well as various enzyme-linked immunosorbent assay (ELISA) techniques (Hands, 1989; Masolun *et al.*, 1992; Jacobs *et al.*, 1995).

The acid production test (see later) can also function as a simple, albeit slower, means of checking that a sample of milk will support a yoghurt fermentation and Hawronskyj *et al.* (1993) have proposed that the ATP bioluminescence procedure could be used as an alternative. However, it is unlikely that either of these latter approaches will replace the commercial systems that are available on the market.

10.4.2 Milk powder

Although process milk can be concentrated by evaporation or ultrafiltration (UF), raising the total solids of the milk base through the incorporation of a milk-based powder is still widely practised in small dairies. In some places, skimmed milk or full cream milk powder may be the only feasible raw material, but whatever the precise role of the powder, an examination of each consignment to ensure its adherence to agreed specifications can avoid problems at a later stage. Standard methods for monitoring the solubility of a milk powder and the production of sediments are well established, and the moisture and fat contents of a powder can likewise be recorded by the agreed procedures of the American Dairy Products Institute (ADPI, 1990) (previously the organisation was known as American Dairy Milk Institute (ADMI); see also Chapter 2 and IDF (1992b) for other specifications of milk powders.

Each consignment must also be tested for antibiotics and a microbiological

Table 10.3 Some suggested specifications for spray dried milk powders to be employed in the production of yoghurt

Standards	Satisfactory	Doubtful	Unsatisfactory
Microbiological			
Total colony count	<10 000	<100 000	>100 000
Coliforms	<10	<100	>100
Yeasts	<10	>100	<100
Moulds	<10	>100	<100
Staphylococci (coagulase positive)	<10	<100	>100
Chemical			
Acidity:	Acidity of reconstituted skimmed milk powder (9 g TS 100 g^{-1}) should not exceed 0.15% lactic acid (see also IDF, 1981b).		
Solubility:	Sediment in the solubility index tube (ADPI, 1990) produced by 10 g of skimmed milk powder should not exceed 0.5 ml (see also IDF, 1982, 1988a)		
Scorched particles:	Employing the apparatus specified in BSI (1982), the filter disc should conform to Disc B of the ADPI photographic standards.		
Moisture content:	Moisture content of skimmed milk powder should not exceed 4.5 g 100 g^{-1} (see also IDF, 1993c).		
Fat content:	Fat content of skimmed milk powder should not exceed 1.25 g 100 g^{-1}.		
Inhibitory substances:	Powder should not contain above 0.006 IU g^{-1} of inhibitory substances.		

examination covering the groups of organisms suggested by Davis and Wilbey (1990) should be routine. Some proposed specifications are indicated in Table 10.3 and with good manufacturing practice, there is no reason to suppose that these standards cannot be attained. The yoghurt manufacturer is fortunate, however, in that the process milk does receive a severe heat treatment (e.g. 85°C for 30 min or equivalent) and hence some latitude with respect to the microbiological quality of the milk powder can be tolerated. The same margin of freedom applies to the stabilisers or other ingredients added prior to heating, but materials incorporated into the finished yoghurt (e.g. fruit and flavouring/colouring agents) need to be monitored with particular care. Unpasteurised fruit, in particular, can prove to be a troublesome source of yeasts or moulds and, in any yoghurt that contains sucrose, fungal infections can rapidly lead to spoilage and consumer rejection. The importance of this aspect can be judged from the standards proposed for some typical fruits (see Table 10.4) and any additional natural or artificial flavours should achieve at least the same specifications. Sucrose can also on occasion act as a source of yeasts and moulds and although rarely a source of infection, its presence should not be forgotten if spoilage problems should arise; osmophilic yeasts can even survive in some of the syrups employed for fruit yoghurts. Success or otherwise in this area can be judged in relation to the microbiological standards proposed for the end product (see Table 10.11), since failure at this latter point can often be traced to faulty ingredients. A further, and sometimes unexpected, source of contamination can be the bulk starter and an additional function of quality control centres on the provision of a viable, clean culture.

Table 10.4 Typical microbiological specifications that can be applied to some additives employed in the manufacture of yoghurt

Product/ organisms	Count ($cfu\,g^{-1}$)
Fruits	
Moulds	<10
Yeasts	<10
Total count	<1000
Coliforms	negative
Other ingredients including chocolate:	
Moulds	<10
Yeasts	<10
Total count	<2000
Coliforms	negative

After Spinks (personal communication).

10.4.3 Starter cultures for standard yoghurt

10.4.3.1 Microbiological examination

The type of starters available have been discussed earlier, but one popular material for inoculation of the production vessels is still a liquid culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in the ratio of 1:1 (chain:chain). In practice, this requirement means checking the balance by direct microscopic examination and, if the count is made quantitative as well (i.e. with a breed smear technique) then the total count for each species should confirm that the culture is suitable for use.

If the number of bacteria is too high to be counted directly, then a 10^{-1} dilution in quarter-strength Ringer's solution can be made prior to preparation of the slides (Robinson and Tamime, 1976). If the sample is agitated for 30 s before the 0.01 ml aliquot is removed, then the areas of the slide (1 cm^2) should contain a countable number of bacteria. Staining with Newman's stain or, after defatting, with methylene blue (Cooper and Broomfield, 1974) or Gram's stain (Davis *et al.*, 1971) is a useful aid to differentiation and for routine purposes, the number of fields to be examined can be reduced from the figure required, in theory, to give an accurate count (Wilson, 1935; Wang, 1941). Thus, Tamime (1977) found that counting ten fields in a five by five cross-pattern overcame uneven spreading and a reasonable estimate of the cell count ml^{-1} of a starter culture could be obtained. The only adjustment required was in relation to the expected ratio, because the chains of streptococci tend to breakdown into small units of two or three cells during dilution. If each one of these units is recorded as "one", then the ratio of streptococci:lactobacilli rises to around 2.7:1 and this ratio has been found to be repeatable with cultures incubated at 42°C.

An alternative technique for obtaining information about the ratio between the two organisms in a starter culture, or in the retail product for that matter, is the total colony count using a medium that selects for one or other species, or differentiates between them on the same plate. Obviously viable counts are more time

consuming than microscopic counts, but they do offer the advantage of recording only viable colony forming units and for the most part these units can be equated with individual cells. The fact that dilution and plating will have broken most of the chains necessitates a modification of the expected ratio, and figures of 5–10 *Streptococcus* : one *Lactobacillus* may well become the accepted norm; the chains of streptococci counted as one in the clump count tend to be longer than the chains of lactobacilli.

A selection of possible media is shown in Table 10.5 and the final choice will probably reflect the preference of the individual operator. However, it is important that different strains of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* will behave differently in the same medium and the performance of Lee's medium is a case in point (Ghoddusi and Robinson, 1996). Thus, while some strains of *L. delbrueckii* subsp. *bulgaricus* will give white colonies, others produce colonies that are identical to those of *S. thermophilus*. Lee *et al.* (1974) suggested that the acid-producing capacity of *L. delbrueckii* subsp. *bulgaricus* was the critical factor and hence that their medium should only be employed for monitoring a starter culture once its performance had been tested; as shown in Table 10.6, L-S differential medium and modified lactic agar (Matalon and Sandine, 1986) are other media

Table 10.5 Some of the differentiating media which can be employed to enumerate *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* from yoghurt or starter cultures

Culture medium	Micro-organism	
	S. thermophilus	*L. delbrueckii* subsp. *bulgaricus*
Hansen's yoghurt agar	High mass colonies, 1–3 mm	Low mass colonies, 2–10 mm
LAB	Smooth colonies	Irregular, hairy or rough colonies
Lee's medium	Yellow colonies	White colonies
L-S differential medium	Round red colonies with clear zone (<0.5 mm)	Irregular, red colonies with opaque zone (>1.0 mm)
Modified lactic agar	Small red colonies	Large, white colonies
Reinforced clostridial medium with Prussian blue	Pale blue colonies with thin, blue halo	Pale blue colonies with wide, royal blue balo
TYP-HGME agar	Small light blue colonies	Large dark blue colonies
YGLP-YL agar	Small brilliant white colonies	Large white colonies
Tryptose proteose peptone yeast agar with eriochrome dye	Oval colonies convex (1–3 mm) opaque white/violet often with a dark centre	Transparent, diffuse colonies (4–6 mm), unidentified shape with an irregular edge
Tryptose proteose peptone yeast agar with Prussian blue	Pale blue colonies with thin, blue halo	Small, shiny white colonies with wide royal blue halo

Note: these media may NOT be selective against other thermophilic lactic acid bacteria and not all strains of *S. thermophilus* or *L. delbrueckii* subsp. *bulgaricus* will give typical reactions.

Data compiled from Lee *et al.* (1974), Eloy and Lacrosse (1976), Robinson and Tamime (1976), Driessen *et al.* (1977), Johns *et al.* (1978), Bracquart (1981), Matalon and Sandine (1986), Millard *et al.* (1989), Bridson (1990), Sanches-Banuelos *et al.* (1992), Onggo and Fleet (1993), Ghoddusi and Robinson (1996) and Graciela Briceno and Martinez (1996).

Table 10.6 Relative performance of some media[a] employed under conditions specified by the original author(s) to enumerate the species in a standard yoghurt culture

Medium	Differential count[b]	
	Lactobacillus	*Streptococcus*
Lee's medium	1300	5000
TPPY agar	300	2700
TPPYPB agar	200	3000
Modified lactic agar	200	1200
L-S differential medium	(48)	
RCPB agar	500	3200
Elliker's agar	(900)	
M17	–	560
Acidified MRS	105	–

[a] Further details of the media are given in Table 10.5. [b] All figures as cfu $\times 10^6$ ml^{-1} of yoghurt, and are overall means from four separate trials (duplicate plates); there were no significant differences between the trials.

( ), Figures in brackets indicate that no differentiation was observed. –, no growth at dilutions used (10^{-6}–10^{-8}).
After Ghoddusi (1996).

that appear to give different responses according to the strains of bacteria under examination.

This problem of strain reaction is also evident in the data shown in Table 10.6, in that recovery from the same culture did on occasions differ by a factor of ten. However, tryptose proteose peptone yeast (TPPY) agar with eriochrome black gave good differentiation, as did reinforced clostridial prussian blue (RCPB) agar and, on both of these media, recovery (confirmed by Gram staining of selected colonies) was good; extremely clear definition was achieved by incoporating Prussian blue into TPPY (TPPYPB) agar in place of eriochrome black T (Ghoddusi and Robinson, 1996; Rybka and Kailasapathy, 1996).

While a single differentiating medium may be preferred for visual counts, the introduction of automatic colony counters may necessitate a change to the use of a medium selective for only one species (e.g. M17 agar for *S. thermophilus* (IDF 1988b, 1991f; Jordano *et al.*, 1992)), or one that gives a total colony count for all organisms of starter origin; a typical selection of such media is shown in Table 10.7. However, it should be noted that even laser counters are prone to error (e.g. there may be clusters of colonies close to the margin of the Petri dish) and that selective media are not always entirely inhibitory of other organisms. For example, acidified MRS agar can support the growth of yeasts and, although the difference in colony morphology is evident to the human eye, the electronic system will record just one total count, a point that could be important if the same medium is employed to monitor total viable counts of starter bacteria in a sample of commercial yoghurt (see also IDF, 1992c, 1997c).

10.4.3.2 Activity tests

The essential characteristic of a good starter (i.e. liquid type) for yoghurt is that it should produce the desired level of lactic acid within a given time. A simple test for this characteristic involves:

Table 10.7 Some of the media which can be employed to enumerate either *S. thermophilus* or *L. delbrueckii* subsp. *bulgaricus* as individual species from yoghurt or starter cultures

Culture medium	Micro-organism	
	S. thermophilus	*L. delbrueckii* subsp. *bulgaricus*
Eugon	No growth	Growth
Lactic agar (low pH)	No growth	Growth
M17	Growth at pH 6.8	No growth
Microassay	Growth	No growth
MRS medium (acidified)	No growth	Growth
Streptosel agar	Growth	No growth
Trypsin digest agar	No growth	Growth
Trypticase soy agar	Growth	No growth
TGV + Na-acetate	No growth	Growth
Elliker's agar[a]	Growth	Growth

[a] This medium is selective for both *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in the presence of casual contaminants and can be useful to check the performance of one of the selective media (see Table 10.5).

Note: these media may NOT be selective against other thermophilic lactic acid bacteria.
Data compiled from Elliker *et al.* (1956), de Man *et al.* (1960), Robinson and Tamime (1976), Shankar and Davies (1977), Driessen *et al.* (1977), Johns *et al*. (1978), Eloy and Lacrosse (1980, 1982), Hamann and Marth (1984a, b), Ogihara *et al*. (1985), Reuter (1985), Bridson (1990), Anon. (1991), IDF (1991f), Sinha (1991), Sanches-Banuelos *et al*. (1992), Onggo and Fleet (1993), Espinosa *et al*. (1994) and Graciela Briceno and Martinez (1996).

Note that other methods for differential enumeration of the yoghurt starter cultures have been reported by Coker and Martley (1982), Radke-Mitchell and Sandine (1984), Peral de Portillo *et al*. (1988), Bracquart and Sagnard (1989), Camaschella and Cislaghi (1989), Millard *et al*. (1989, 1990), Neviani *et al*. (1992), Ehrmann *et al*. (1994), Pirovano *et al*. (1995), Yamani and Ibrahim (1996), and Dave and Shah (1996).

- making a 1:10 dilution of the starter with 9 ml of Ringer's solution (1/4 strength) or peptone solution;
- placing 10 ml of process milk into a test tube and adding 1 ml of diluted starter;
- incubating the inoculated milk for 4 hours at 42°C.

At the end of this time, the acidity of the milk should be around 0.85–0.95% lactic acid and any cultures that fail to achieve these figures should be regarded with suspicion. This concern stems from the fact that, with a system of daily starter propagation, the balance between the organisms can change over a number of transfers. During manufacture, this swing may be manifest in a number of undesirable ways and an early warning of impending problems, gained through this simple activity test, can be helpful (IDF, 1991f, 1997c; Anon. 1995a).

10.4.3.3 Absence of contamination

The presence of gas bubbles in a liquid type starter culture or an unclean smell are clear indications of gross contamination and a useful confirmatory test is the catalase reaction. Thus, the starter organisms are catalase negative, so that if 5 ml of a culture are added to 1 ml of hydrogen peroxide (10 v), the formation of gas bubbles indicates a considerable infection by non-starter bacteria.

If the starter is being propagated on a daily basis, then a routine examination for coliforms may be worthwhile for, although the high acidity should restrict their survival, slow acid development can allow sufficient buildup to give taints or off-flavours to the retail product. The straightforward test for "acid plus gas" in single strength MacConkey broth is usually adequate for this purpose and if three tubes of broth are inoculated at three consecutive dilutions of the starter (e.g. 10^{-1} down to 10^{-3}) an indication of numbers of presumed coliforms can be obtained; "absent in 1 ml of starter" should be regarded as the minimum acceptable standard.

Although an examination for coliforms can be helpful, if only as an indicator of poor hygiene, the presence of yeasts or moulds at $>10\,\text{cfu}\,\text{ml}^{-1}$ of starter is likely to lead to spoilage during the shelf life of the retail product. Contamination of this magnitude can be readily monitored using malt extract agar acidified with lactic acid or chloramphenicol agar (IDF, 1990d) and a 10^{-1} dilution of the starter is convenient for incorporation into pour plates (1 ml per Petri dish). This approach should, at least, indicate if yeasts are present but, if the original counts are $<100\,\text{cfu}\,\text{ml}^{-1}$, it may be necessary to dispense 1 ml of undiluted culture into three standard Petri dishes (9.0 cm diameter) or one large dish (14 cm). Particular attention should be paid to any signs of infection by species capable of utilising lactose (e.g. *Kluyveromyces marxianus* var *marxianus* or var *lactis*) and their presence must be regarded as a stimulus for immediate action, namely improvements in the hygiene of the culture facility and the propagation of a fresh mother culture.

These routine examinations of bulk starters are essential where culture maintenance is carried out on-site and if the necessary laboratory facilities are not available, then consideration should be given to the use of freeze-dried or deep-frozen cultures for direct inoculation of the bulk starter milk. Thus, the cultures available from commercial manufacturers have an excellent record in respect of freedom from contamination and overall performance and the yoghurt manufacturer can normally be excused the rigours of a detailed starter examination (see also IDF, 1988b, 1991f, 1992c, 1997c).

10.4.4 Starter cultures for bio-yoghurts

While bulk starter cultures are still used for the production of normal yoghurt, the cultures for bio-yoghurts are usually of the concentrated freeze-dried or deep-frozen, direct-to-vat inoculation (DVI) type. The reason for this contast is that: (a) *Lactobacillus acidophilus*, *Lactobacillus paracasei* subsp. *paracasei, Lactobacillus reuteri*; *Lactobacillus rhamnosus*, *Lactobacillus paracasei* biovar *shirota* and *Bifidobacterium* spp. are difficult to grow in milk, and it is difficult to maintain the ratio(s) in a bulk starter if grown as a mixed culture, and (b) the end products must have viable cell counts above the agreed therapeutic minimum (Robinson, 1989; Marshall and Tamime, 1997). By using a DVI culture with a known cell count, the manufacturer is able to calculate with some accuracy the incubation time necessary to obtain the desired final counts and, equally important, can have confidence that those same counts will be achieved day after day. For this reason, the manufacturer of a bio-yoghurt will rely on the specification from the culture supplier and any microbiological checks are made on the end products instead.

10.5 Quality appraisal of retail products

However advisable it may be to monitor standards of plant hygiene or to insist that raw materials meet agreed specifications, it is the end product that must pass the final test – does it meet any legal requirements and is the quality acceptable to the consumer? In some countries, the imposition of compositional standards aims to encourage the maintenance of quality but, for the most part, the nature of the product in terms of consistency and related features ensures that the proposed standards are met with little difficulty. Nevertheless, analysis of the end product is an essential feature of quality control, because problems in manufacture are almost certain to manifest themselves as faults in the product. Consequently, examinations at this stage:

- protect the consumer from the purchase of poor quality product or, in extreme cases, product that might constitute a health hazard;
- protect the manufacturer from the inconvenience and expense of a barrage of returned goods;
- assist in the smooth operation of a plant by identifying variations in product quality at an early stage, so that any necessary corrective actions can be taken before the onset of serious problems.

The appraisal of product quality has become, therefore, a vital function of factory operation, and the gamut of examinations that may be performed can be considered under the headings that follow (see also Brant, 1988).

10.5.1 Analysis of chemical composition

Many countries have legal standards, or at least provisional regulations, for example MAFF (1975) and DTF (1983) in the UK, covering the composition of yoghurt and a selection of the existing proposals is given in Table 10.8 (see also IDF, 1984; Anon., 1986, 1987c, 1988a; Kirihara *et al.*, 1987; FAO/WHO, 1990). The requirement for a value for SNF is, in reality, more decorative than essential, because the texture or viscosity of a natural yoghurt with an SNF below the stipulated minimum would be barely acceptable. An overall measurement of total solids could, however, be valuable as a check that the concentration or fortification has been carried out correctly and a modification of the standard gravimetric method for milk has been proposed (Kirk and Sawyer, 1991) as suitable for yoghurt. The sample is neutralised before drying with 0.1 N strontium hydroxide and 0.0048 g ml^{-1} of alkali is deducted from the dry weight of the sample (see also Dordevic *et al.*, 1990). Davis and McLachlan (1974) suggested the use of vacuum drying with sodium hydroxide as the reagent. Either technique provides a convenient method of monitoring total solids; drying samples in a microwave oven did not appear to be satisfactory for yoghurt (Marquez *et al.*, 1995).

The routine measurement of protein is essential in large dairies because, over a typical year, the protein content of cow's milk may vary from 3.2 to 3.6 g 100 g^{-1}, and these differences are enough to alter the quality of the yoghurt. The cost of standardisation is, therefore, acceptable for a plant using several million litres of milk per week and the Kjeldahl method (IDF, 1985, 1990c, 1993b) remains the reference method for total nitrogen/protein in milk. Although the Kjeldahl remains the standard method, Karman and van Boekel (1986) have questioned whether 6.38 is the

Table 10.8 Some reported standards for the chemical composition (g $100g^{-1}$) of yoghurt in terms of milk fat and solids-not-fat (SNF)

Country of origin	Types of yoghurt based on fat				
	Strained	Normal	Medium	Low	SNF
Argentina	–	2.8	0.5	0.5	–
Australia	–	3.0	0.5–1.5	0.5	–
Belgium	–	3.0	1.0–3.0	1.0	8.2
Denmark	–	3.5	1.5–1.8	0.3	–
FAO/WHO	–	3.0	0.5–3.0	0.5	8.2
Finland	–	2.5	–	–	–
France	–	3.0	–	1.0	–
Germany	10.0	3.5	1.5–1.8	0.3	–
Greece	5–8	5.0	–	–	–
Israel	–	3.0	1.5	0.2–0.5	–
Italy	–	3.0	1.5–2.0	1.0	–
Kenya	–	2.25	–	1.25	8.5
Kuwait	–	3.0	0.5–3.0	0.5	–
Lebanon	–	3.0	–	–	8.2
Luxembourg	10.0	–	–	–	9.0
Netherlands	4.4	2.95–4.4	1.5–1.8	0.5	8.2–12.6
New Zealand	–	3.25	–	2.0	–
Portugal	–	3.0	0.5–3.0	0.5	8.2
Spain	–	2.0	–	–	0.5
Switzerland	–	3.0	1.5–2.5	0.5	–
Sweden	–	3.0	–	–	0.5
South Africa	–	3.3	1.5–2.5	0.1–0.5	8.3–8.6
U.K.	–	3.5	1.0–2.0	0.3	8.5
U.S.A.	–	3.0–3.8	2.0–2.8	1.0–0.5	8.25–8.3

Adapted from Robinson and Tamime (1976); Australian Standards (1978), Anon. (1977, 1983, 1988a, 1996) and Kuwait Standards Specifications (KSS, 1980).

most appropriate factor. Whatever the validity of this point, there is no doubt that it is a time-consuming procedure and most dairies rely on measurements of the infrared absorption spectra (Anon., 1987a; IDF, 1990c; AOAC, 1990; Andersen *et al.*, 1993). The advantages of this approach are that it measures true protein, unlike the reference method which includes the non-protein nitrogen fraction and the infrared absorption technique can be applied to the measurement of fat, lactose and water, so that one instrument can give an accurate and rapid analysis of all the relevant components (Briggs, 1979). Obviously the calibration has to be established for the type of material to be analysed, for example, the incoming milk or the yoghurt as it leaves the vat, but modern instruments can provide full analysis for up to 360 samples per hour.

The other significant component, namely fat, is of interest not only in relation to any legal standards, but also because: (a) many stirred yoghurts are designated to be low or very low fat and hence it is important that the description should not be misleading, (b) milk fat has a major impact on the mouthfeel of yoghurt, around 1 g $100\,g^{-1}$ being regarded as the minimum to produce the desired response from the consumer, and (c) it is anticipated that full fat natural yoghurt (3.0–3.5 g $100\,g^{-1}$), 'luxury' fruit yoghurts (>4.0 g $100\,g^{-1}$) and Greek-style yoghurts (>8.0–10 g $100\,g^{-1}$) will have high fat contents and again these expectations must be met.

The gravimetric methods of determining fat in yoghurt (e.g. the Röse Gottlieb method) are regarded as the most accurate (Davis, 1970) but for routine purposes, the normal Gerber method (BSI, 1989a; IDF, 1997a) using 11.3 g of yoghurt in a milk butyrometer is totally appropriate. All these examinations should be performed on the natural yoghurt prior to the addition of fruit and monitoring the acidity is also more straightforward in the absence of additives. However, while the Röse Gottlieb method remains the reference method (IDF, 1988c), fat is routinely monitored by light scattering photometry (Anon., 1987a) or within a multicomponent analysis using infrared absorption (Andersen *et al.*, 1993).

The production of lactic acid beyond the point of coagulation is monitored principally in relation to consumer preference and hence the selected end point will vary not only from country to country, but also with the type of yoghurt. Thus, in The Netherlands, for example, Bulgarian yoghurt may have an acidity of up to $1.48\,g\,100\,g^{-1}$ lactic acid, while other types are usually sold with a maximum of $1.17\,g\,100\,g^{-1}$ lactic acid (Netherlands Standards, 1967). The IDF (1992c) have suggested a minimum of 0.7 g lactic acid per 100 g of retail product and hence the measurement of acidity is an important feature of production. Although the configuration of the lactic acid can be important from a nutritional standpoint, it is usually assumed that culture selection will determine whether the D (–) or L (+) isomer will dominate (see also Anon., 1995b). However, in situations where the characteristics of the culture are not known, there are colorimetric methods available (Lunder, 1972) to determine the total level of lactic acid and, subsequently, of the L (+) isomer and HPLC can achieve the same separation (Olieman and de Vries, 1988); an enzyme-based biosensor could also be used to identify L (+) lactic acid specifically in yoghurt (Mulchandani *et al.*, 1995).

Although the relationship between titratable acidity and pH is not straightforward in a highly buffered system like yoghurt (Lück *et al.*, 1973), the direct electrometric determination of pH is extremely convenient (Harrison *et al.*, 1970). Thus, once a correlation has been established between pH and the desired characteristics of a particular type of yoghurt, then routine monitoring during manufacture can become a normal practice. However, to maintain a close check on the acidity of the retail product, it is usually desirable to test representative samples of the cooled yoghurt for titratable acidity.

The measurement is a composite one including the natural acidity of the milk and the developed acidity arising from bacterial activity but, as the natural acidity should not vary a great deal (assuming that the milk is standardised for total solids), titratable acidity is a reasonable indication of the performance of the starter culture. The problems of measuring acidity by direct titration have been discussed by Sherbon (1988) and, for the analysis of yoghurt, the approach is based on the technique employed for liquid milk. Thus, the normal method involves transferring a known volume or weight of natural yoghurt to an evaporating basin and then neutralising the acidity with caustic soda. A detailed summary of some of the suggested methods is shown in Table 10.9 and it is noticeable that the expression of results differs from country to country. In practice, these national preferences are not important but, for comparative purposes, a chart of the type shown in Appendix I can always be constructed.

The subjective nature of the end points is more relevant, because it implies that some variation between operators has to be accepted, and hence a comparison of results from different laboratories may not always be possible. It also means that in

Table 10.9 Selection of methods for determining the titratable acidity of milk or fermented milk

Component	BSI (1989b)	Danish[a]	Netherland[a]	Tamime (1977)	IDF (1991h)	AOAC (1990)
Sample size	10 ml	25 ml	10 ml	10 g	10 g	20 ml or g
Dilution	–	–	–	–	–	2:1
Phenolphthalein[b]	1 ml	13 drops	0.5 ml	1 drop	–	2 ml
Alkali[b] (M NaOH l^{-1})	0.1	0.1	0.1	N/9	0.1	0.1
End point[b]	Pink to match cobalt (II) sulphate or reference colour solution	Constant pale red colour	Pink to match fuchsin standard	Light rose to persistent pink colour	Titrate to pH 8.3	First persistent pink colour
Expression of results	Alkali (ml) 10 = g lactic acid 100 ml^{-1}	Alkali (ml) × 4/100 ml	1/10 ml of alkali ml^{-1}	Alkali (ml) 10 = % lactic acid or g lactic acid 100 g^{-1}	Alkali (ml) × 0.9 10 g = g lactic acid 100 g^{-1}	Alkali (ml) 20 = % lactic acid or ml alkali 100 g^{-1}

[a] Adapted from Robinson and Tamime (1976). [b] For the preparation of reagents refer to standards.

Note: For conversion of degrees Dornic (°D), Thorner (°T) or Soxhlet-Henkel (°SH) to % lactic acid refer to Appendix I.

any given laboratory, the measurement of titratable acidity should be carried out under standardised conditions, that is, a specific location in the laboratory with a non-variable light source, and that the actual titration should be performed by the same person. If these restrictions can be met, then titratable acidity becomes a most useful measurement, because not only can the figures be linked fairly accurately to consumer preferences but, through the component for developed acidity, changes in performance of the starter bacteria can be manifest rapidly.

Monitoring of other milk components like lactose is probably not important as a routine, but Mistry *et al.* (1989) can be consulted for a list of available methods; sucrose levels in the milk base or the final product can be checked instrumentally (Anon., 1981). The introduction of legislation covering additives and colouring materials means that close inspection of ingredient specifications (see Table 10.10) and/or additional specialised analyses are required. Thus, the addition of a fruit puree containing starch to a yoghurt base already incorporating a compound stabiliser could raise the total starch above the suggested $1\,g\,100\,g^{-1}$ level, and preservative levels would need to be similarly monitored, at least on an occasional basis. The timing and extent of such analyses will differ from company to company and standard texts, such as AOAC (1990) or Kirk and Sawyer (1991), should be consulted concerning appropriate methods and their application.

10.5.2 Assessment of physical characteristics

Yoghurt is normally retailed in one of three physical states, namely set yoghurt, stirred yoghurt and fluid or drinking yoghurt, and each type has quite distinctive characteristics. The typical gel structure of a set type, for example, could never really be mistaken for the semifluid form of the stirred variety, but the low viscosity of some stirred brands leaves the consumer with little option but to drink them. This degeneration of product image is obviously regrettable and, although the release of an occasional poor batch is inevitable, the question of desirable viscosity is always somewhat vexing. In practice, each manufacturer will probably adopt an agreed in-house standard for viscosity (or consistency in the case of set yoghurt) and then operate to this specification, so that the routine assessment of these physical features becomes a normal part of quality control.

10.5.2.1 Set yoghurt

The essential gel structure of set yoghurt means that assessment of the product must be approached in a manner that does not destroy the delicate coagulum. The falling sphere technique (Pette and Lolkema, 1951) can be used, but the most convenient test involves the use of the penetrometer (Hartman, 1976; see Fig. 10.3). The only special adaptation centres on the choice of spindle and cone, for these have to be selected so that, for the product in question, the depth of penetration of the cone does not exceed about 33% of the total depth of the retail sample. The risk of edge effects from the carton must be minimised by choosing a spindle with a diameter no greater than 50% of the diameter of the pot and, with these restrictions in mind, it becomes a simple matter to select a probe/spindle weight that is appropriate. The configuration of the probe (i.e. flat or cone-shaped) is also variable, so that products with 12–16 g TS $100\,g^{-1}$ can be handled with ease. In addition, the weight of the probe/spindle may be changed in relation to temperature of the product, for example, a light spindle might be selected for examination of a carton at 42°C

Table 10.10 Some proposed or existing regulations concerning the introduction of non-dairy ingredients into stirred fruit yoghurts; no additives are usually permitted in natural set or stirred yoghurts

Country	Stabiliser	Fruit	Preservatives	Colours/flavours
Belgium	Gelatin Starch/modified starch[a] Pectin	–	Sorbic acid and salts	Anthocyan, caramel, carotenoids, chlorophyll, cochineal Xanthophyll Vanilla extract, ethyl vanillin
Denmark	nil	10–15%	nil	nil
France	–	Up to 30%	–	Anthocyan E 163, beet root red E 162, caramel E 150, carbo medicinalis vegetalis E 153, carotinoid E 160, chlorophyll E 140, cochenille E 120, curcumin E 100, indigotine E 132, lactoflavin E 101, xanthophyll E 161
Greece	nil	–	nil	nil
Italy	–	Up to 30%	Sorbic acid E 200 Sodium, potassium and calcium sorbate	Specified colours and listed preservatives may only be added with the fruit
Luxembourg	Permitted	<8%	Sorbic acid and salts	As with stabilisers, permitted from positive EU lists
Netherlands	Permitted	–	–	Permitted
Portugal	–	–	Sorbic acid and potassium sorbate	Carotene E 160a, bixin E 160b, cochenille – colours and preservatives may only be present in the fruit
United Kingdom	Starch[a] Pectin Gelatin Alginates[b] Agar Edible gums Celluloses	Up to 30%	Sulphur dioxide Benzoic acid Methyl-4-hydroxybenzoate Ethyl-4-hydroxybenzoate Propyl-4-hydroxybenzoate Sorbic acid	

[a] Any combination not to exceed 1.0%. [b] Any combination not to exceed 0.5%.

After: Robinson (1976) and Anon. (1989).

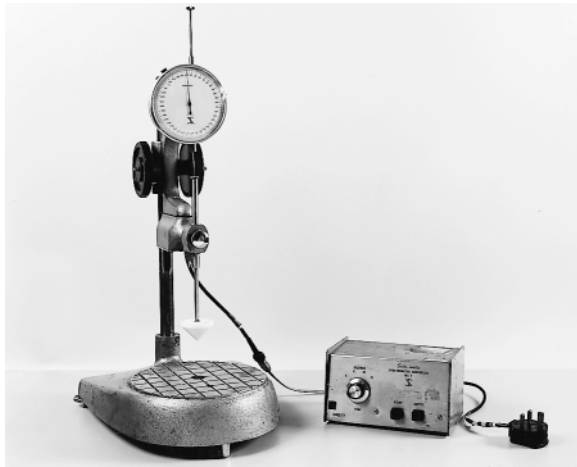


Fig. 10.3 Standard penetrometer of the type that can be employed to measure the consistency of set yoghurt

immediately after incubation and a heavier spindle for assessment of the firmer coagulum developed in yoghurt held subsequently at 7°C for 24 hours. These changes in spindle weight make it possible to discriminate, at a given temperature, between samples of different gel strengths and the fact that comparisons are possible at 42°C makes it feasible to predict the consistency of the retail product prior to final cooling.

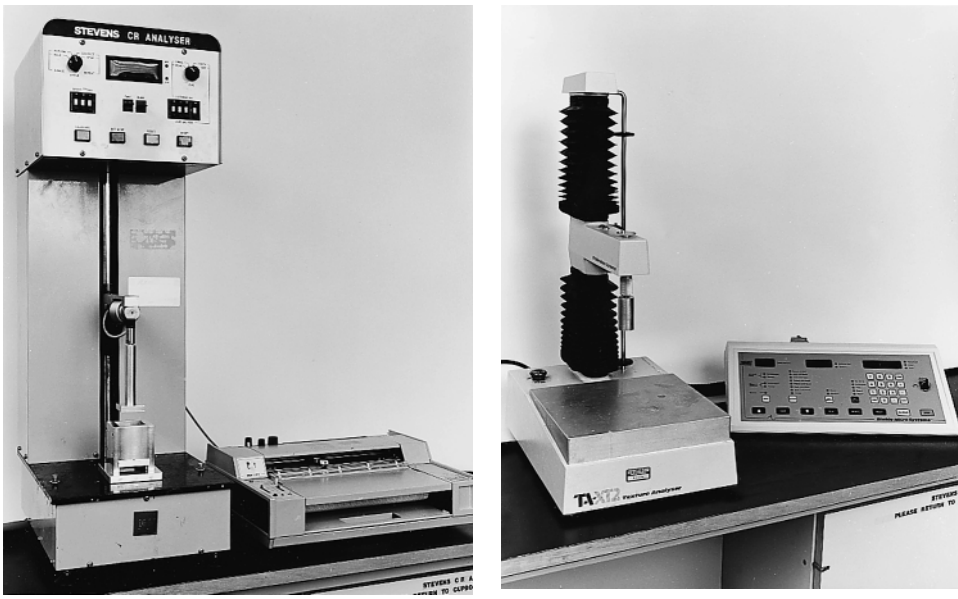
The technique is, therefore, both reliable and versatile and hence standardising the physical properties of set yoghurt becomes a straightforward exercise. If the data are required for research and development purposes, the use of a computerised texture profile analyser (TPA) (see Fig. 10.4 A and B) may improve the repeatability of the measurements (Prentice, 1992; Benezech and Maingonnat, 1993, 1994; Vélez-Ruiz and Barbosa Cánovas, 1997). Other physical features or faults, for example lumpiness or the presence of nodules, usually become apparent during sensory analysis. These problems will be discussed later.

10.5.2.2 Stirred and fluid yoghurt

The range of methods that are available to measure the viscosity of fluid and semi-fluid products has been discussed by Sherman (1970) and Prentice (1992) and the choice of method is really a matter of operator preference. Thus, in the present context, interest centres on making an objective comparison between samples, or between a sample and an expected result representing product of acceptable quality. A number of simple techniques can be employed for this purpose.

At one end of the scale, some small producers rely on extremely simple techniques, such as:

- scooping a sample of yoghurt onto the back of a spoon, and then gently inclining the spoon downwards – the rate at which the yoghurt drips from the spoon is a reflection of its viscosity; the same technique will also reveal any irregularities in the coagulum;



(A) (B)

Fig. 10.4 Texture profile analysers (A: Stevens compression response analyser; B: TA-XTA texture analyser) suitable for measuring the firmness of yoghurt

- inserting a plastic teaspoon into a typical retail carton of yoghurt – if the spoon remains upright, the product has an acceptable, spoonable viscosity;

and, although these approaches are subjective in the extreme, they do offer the experienced operative a guide to the quality of the end product.

It is more usual, however, to rely on more reproducible techniques and a number of these are available. Thus, Davis (1970) has described the use of a rotating cylinder which could be tilted until the yoghurt began to pour; the angle necessary to initiate flow can be taken as a measure of product viscosity. The time taken for a standard metal sphere to descend a certain distance through a prescribed volume of yoghurt has also provided a convenient method of comparison (Ashton, 1963; Bottazzi, 1976), as has the flow rate of yoghurt through funnels of prescribed orifice sizes; in the Posthumus funnel, for example, the time taken for the yoghurt "surface" to pass between the starting point and the centrally located pointer gives a measure of the viscosity of the product (Posthumus, 1954). A similar approach has been employed in The Netherlands (Galesloot, 1958), in South Africa (Ginslov, 1970) and in Sweden (Storgards, 1964). The time taken for a yoghurt sample of known volume to flow down an inclined plane, with or without weirs, has also been advocated, as has the "plummet" (Hilker, 1947), but perhaps the most universally accepted approach is that employing a rotational viscometer (see Fig. 10.5), or the torsion wire apparatus. Another empirical method used to measure the rheological property of stirred yoghurt is known as the Bostwick consistometer. The unit resembles a rectangular channel made from stainless steel and fitted with removable slot or door. The consistometer can be used on-site in the production area where a sample of yoghurt is placed in the slotted compartment; the door is removed and the rate

(A)

(B)

Fig. 10.5 Rotational viscometers (A: Brookefield Synchro-lectric; B: Haake) that would be suitable for comparing the viscosity of different samples of stirred yoghurt

Note: With the Haake, the geometry of the system is constant and circulating water allows for accurate temperature control.

of flow per time is measured on a scale. The thinner or low viscous yoghurt tends to flow faster (Fig. 10.6).

The ease of operation makes the rotational viscometer, such as the Brookfield Synchro-Lectric, a popular choice, and once the type of spindle and its speed of rotation have been established for a given product, comparison between successive batches presents few problems; the Helipath system in which the spindle rises vertically through the sample while rotating is preferable since there is less risk of the spindle causing local syneresis and an artificially low reading (Abrahamsen and Holmen, 1980; see also Hellinga *et al*., 1986). It is reasonable to stipulate, therefore, that a stirred yoghurt should have a viscosity that falls within certain preset limits and the physical nature of a fluid yoghurt could be similarly described; for thick, stirred yoghurts (e.g. labneh or Greek-style yoghurts) Tamime *et al.* (1989, 1991) have suggested that the use of a Stevens-LFRA texture or compression response analyser should be considered (see Fig. 10.4). Handling batches that fall outside these categories is a matter for company policy, but clearly, monitoring this aspect of product quality can be undertaken on a routine basis.

However, although methods of this type have the speed and simplicity essential for routine quality control, some authorities argue that the actual figures do not reflect the true nature of the product, since the shearing effect of the spindle destroys the integrity of the coagulum. Obviously processing alone has disturbed the original gel of stirred yoghurts but even so, the coagulum does tend to reform to some extent during cooling. Consequently, it has been suggested that as stirred yoghurt is a viscoelastic material, that is, it has some of the properties of a viscous

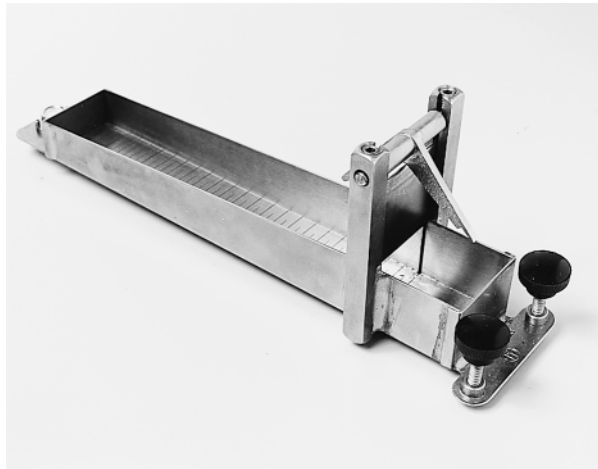


Fig. 10.6 A Bostwick consistometer

liquid and some of an elastic solid (Ozer *et al.*, 1997), dynamic oscillatory testing would be more appropriate (Steventon *et al.*, 1990; Vlahopoulou and Bell, 1990; Xiong and Kinsella, 1991; Rönnegård and Dejmek, 1993). Thus, yoghurt gels are particulate structures composed mainly of caseins (Dickinson, 1990) and, in general, continuously connected strands of protein produce a heterogeneous three-dimensional gel network which holds free water. Any factors which affect the properties of the gel network by changing the nature and number of protein interactions will also affect the water holding capacity of the gel. The gel structure is known to involve both covalent (thiol/disulphide interchange) and non-covalent bonds (Roefs, 1986; Brendehaug, 1987; Mottar *et al.*, 1989; Roefs and van Vliet, 1990; Langton, 1991; Amice-Quemeneur *et al.*, 1995). Dickinson (1994) claimed that the physical characteristics of particulate gels are determined by the strong, permanent (covalent) bonds formed during the aggregation of protein particles. Furthermore, the structure of the final gel is also dependent upon the number of weak, reversible interactions that occur between the particles prior to formation of the permanent bonds. In effect, the numerical balance between the strong and weak bonds controls the rheology of the gel (Dickinson, 1994).

Another factor that affects the physical characterisitics of yoghurt is the distribution of protein–protein bonds over the gel network (Walstra, 1997). A number of studies have investigated the relationship between protein concentration, the distribution of protein–protein bonds and the rheology of the resultant gels (Bremmer *et al.* 1990; Walstra *et al.* 1990). Thus, although in homogeneous gels all the components contribute to the network (Roefs and van Vliet, 1990), in non-homogeneous materials like yoghurt, thick protein "nodes" including more than one protein junction point are evident; the contribution of the protein–protein bonds to the elasticity of the gel decreases as the number of stress-carrying strands is reduced.

When the texture of a set yoghurt is measured with a penetrometer or texture analyser or the viscosity of a stirred yoghurt is determined by one of the conventional techniques (Ozer *et al.*, 1997), the disturbance breaks the chains of casein micelles that form the three-dimensional network which immobilizes the liquid phase (Heertje *et al.*, 1985). However, by examining such a structure with a

controlled-stress rheometer, two parameters which indicate the elastic and viscous characteristics of the gel can be determined with minimal disturbance to the inherent structure.

The Rheotech international controlled-stress rheometer (see Fig. 10.7) is typical of the instruments that can be used to make dynamic measurements; the oscillating surface is a parallel plate of 20 mm diameter. The gap between this plate and the stationary surface is variable but, for a soft gel like yoghurt, a gap of around 10 mm is normal. In practice, the frequency and amplitude ranges of the sinusoidal waves generated by the moving plate are established for the product in question, so that all readings fall within the so-called linear viscoelastic region (LVE) (Ferry, 1980). In this region, both shear moduli are independent of strain and stress and the plateau ends at the applied strain required to cause the material to break down. Some typical conditions for yoghurt are: (a) set yoghurt: frequency 0.25 Hz; amplitude 0.015 mN m (minimum), 0.15 mN m (maximum) and (b) stirred yoghurt: frequency 0.25 Hz; amplitude 0.008 mN m (minimum), 0.08 mN m (maximum) with all measurements being made at 25°C.

Once the conditions have been established, the storage modules G' can be measured. This parameter expresses the energy stored in the material from rearrangements in the structure that take place during the oscillation period; solids tend to return to the original state after a stress is released, and hence G' confirms the elastic characteristics of the product. Then, the loss modulus G'' can be determined and this value records the energy lost during the cycle of deformation, so indicating the viscous component of the material. These measurements should, therefore, give an accurate picture of the gel structure (van Marle and Zoon, 1995).

An example of the application of this technique is shown below and the materials are concentrated yoghurts (23 g TS 100 g^{-1}) produced from normal yoghurt (16 g TS 100 g^{-1}) by the traditional (cloth bag) system, UF (Tamime *et al.*, 1989, 1991) and RO. The protein content (g 100 g^{-1}) of the product derived by the traditional process was 8.00, by UF 8.13 and reverse osmosis (RO) 6.38. As a high protein

Fig. 10.7 Overall view of a rheometer linked to a computer to enable dynamic measurements of yoghurt structure to be made

content leads to an increased number of protein interactions and protein–protein bonds, so the elastic character of the gel (G') should increase and, as shown in Fig. 10.8, the traditional and UF samples had higher storage moduli; the enhanced protein levels made the gels less prone to breakdown with increasing amplitude. Exactly why the traditional product showed increased elastic properties was not determined, but it seems likely that slow drainage under gravity altered some aspect of the protein–protein interactions.

The same increase in concentration of protein causes the space occupied by the protein network to increase, so restricting the mobility of free water; higher values for G'' (viscous component) may be expected (see Fig. 10.9). It is noticeable also that the values for the storage moduli (G') are higher than those of the loss moduli

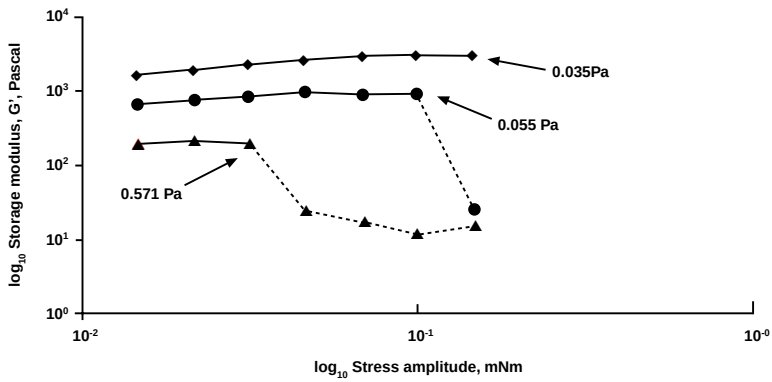


Fig. 10.8 Storage moduli of samples of yoghurt concentrated by the traditional (cloth bag) method (◆), ultrafiltration (●) and reverse osmosis (▲) to 23 g TS 100 g^{-1}; the points indicate the amplitude strain at the stress in question, and the arrows show the position at which structural breakdown was noted

Data compiled from Ozer (1997)

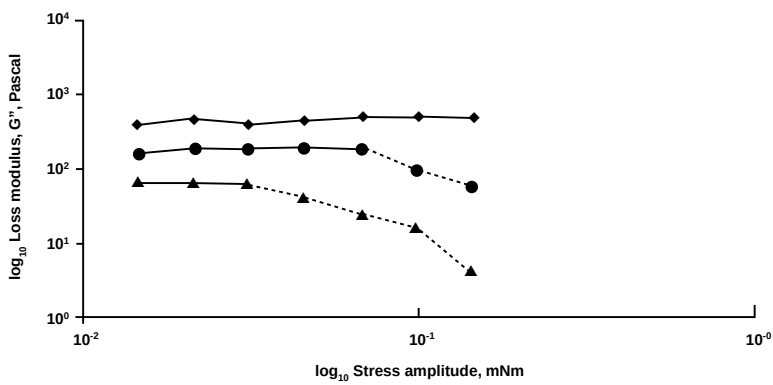


Fig. 10.9 Loss moduli of samples of yoghurt concentrated by the traditional (cloth bag) method (◆), ultrafiltration (●) and reverse osmosis (▲) to 23 g TS 100 g^{-1}

Compiled from Ozer (1997)

over the range measured, so confirming that yoghurt has, as widely reported, a weak viscoelastic structure. In addition, it is fair to assume that the stronger protein bonds contribute to the elastic character of viscoelastic gels, whereas the loss modulus (G'') reflects the number and/or distribution of weak bonds. Both the number and distribution of protein bonds throughout the gel network seem to be dependent on the protein content (Ozer *et al.*, 1997). As the changes in storage moduli (G') as a function of amplitude were paralleled by changes in the values for loss moduli, considerable differences in the loss tangent (tan δ) figures were recorded, especially at the higher amplitudes (see Fig. 10.10). This suggests that the nature of the interactive forces were essentially dependent on the variables examined; the loss tangent (tan $\delta = G''/G'$) is indicative of the nature of the interaction forces in a gel (Ferry, 1980) or the methods used to fortify milk solids with skimmed milk powder (SMP) or UF retentate (Biliaderis *et al.*, 1992) or the level of fat in the milk base (de Lorenzi *et al.*, 1995). However, as shown in Chapter 2 the processing conditions can influence the rheological properties of yoghurt. Some selected studies include: (a) a study shawing that the shear rate and time dependency were influenced by both the presence of pectin and strawberry concentrate (Ramaswamy and Basak, 1991a, b; Basak and Ramaswamy, 1994; Butler and McNulty, 1995), (b) the influence of heat treatment of the milk base on the firmness of the gel, reported by Schmidt *et al.* (1985), Parnell-Clunies *et al.* (1986a, b) and Gebhardt *et al.* (1996), and (c) the rheological properties of yoghurt made with encapsulated non-EPS (exopolysaccharide) cultures or the effect of humectants (e.g. NaCl, sucrose or sorbitol), have been detailed by Hassan *et al.* (1996a, b) and Lacroix and Lachance (1988), respectively. All these rheological studies on yoghurt have been carried out on packaged products, but Picque and Corrieu (1988) and Doi *et al.* (1992) described techniques to make in-line measurements of gel characteristics and to monitor milk curd formation continuously. The inverse photoelectric method had been used to characterise yoghurt through the measurement of its thermal effusivity or measurement of penetration coefficient $(k\rho c)^{1/2}$. The results suggest that the sensitivity of the technique is influenced by the fat : water ratio (Bicanic *et al.*, 1994).

Certainly, dynamic rheometry does expose differences in the rheological charac-

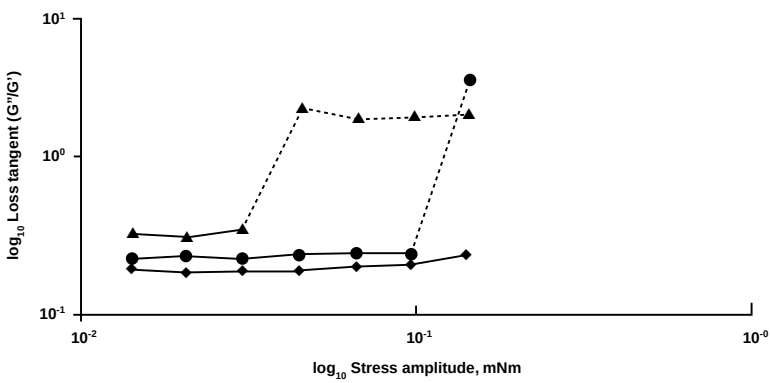


Fig. 10.10 Loss tangent (G''/G' = tan δ) of samples of yoghurt concentrated by the traditional (cloth bag) method (◆), ultrafiltration (●) and reverse osmosis (▲) to 23 g TS $100\,g^{-1}$

Compiled from Ozer (1997)

teristics of stirred yoghurts that would not be apparent on the basis of destructive measurements with a viscometer (Teggatz and Morris, 1990; Rohm, 1992, 1993; Skriver *et al.*, 1993, 1995; Rohm and Schmid, 1993; Rohm and Kovac, 1994; Skriver, 1995; Rawson and Marshall, 1997; Hess *et al.*, 1997; Ozer *et al.*, 1998). However, the disadvantages of this approach are that the equipment (see Fig. 10.7) is expensive in contrast to the cost of a viscometer, and that taking the measurements can be technically demanding, and hence for routine operation in quality control, it is a system that is unlikely to find much application.

10.5.3 Microbiological analysis

While the techniques for measuring physical properties and chemical composition can be applied to any type of yoghurt, a microbiological examination of the finished product may include checks on the survival of the starter organisms, as well as for the presence of undesirable spoilage or pathogenic organisms. The tests for pathogens will, of course, be used for many dairy products but as the specific microfloras of yoghurt and bio-yoghurt may well be different, the two types of product will be discussed separately.

10.5.3.1 Standard yoghurt

Interest in an examination for the bacteria of starter origin stems from the fact that low population levels of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* may be associated with excessively long incubation times and poor development of the typical yoghurt flavour. However, excessively high levels can result in:

- too rapid or excessive acidification,
- syneresis in set yoghurts,
- an imbalance of flavour components,
- spoilage from continued acid production during storage, even at low temperature.

In addition, it has been suggested that "yoghurt should contain abundant and viable organisms of starter origin" (FAO/WHO, 1990) and, whatever the precise definition of these words, there is a general agreement that yoghurt should contain live bacteria unless specifically designated as pasteurised or heat-treated (see also IDF, 1992d). Many countries stipulate also that the term yoghurt should only be applied to a milk fermented with *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* alone (Bourlioux, 1986; Anon., 1989), and there is a continuing debate as to whether the word "yoghurt", alone or qualified, should be used for any product that does not contain *L. delbrueckii* subsp. *bulgaricus*. From an historical perspective, this proposal makes sense, but the framing and enforcement of such a regulation could prove more difficult. For example, Table 10.11 shows some typical figures for starter bacteria in retail yoghurts, and most reports suggest that counts of this order are common (Hamann and Marth, 1984a; Sinha *et al.*, 1989; Roberts and Maust, 1995). The question does arise, however, regarding whether a yoghurt must have counts of this order, or whether a few cells of *L. delbrueckii* subsp. *bulgaricus* ml^{-1} of product will suffice.

The methods available for examining the starter flora of normal yoghurt have been discussed elsewhere (see Starter cultures for standard yoghurt, Section 10.4.3) and an indication of the results that might be anticipated is shown in Table 10.11.

Table 10.11 Indication of the numbers of starter bacteria that have been isolated from retail cartons of yoghurt and some suggested standards relating to both contaminants and desirable organisms

Organism	Yoghurt		
	Natural	Strawberry	Blackcurrant
S. thermophilus $\times 10^6$ cfu ml^{-1}	10–820	35–1100 54–250	80–1850
L. delbrueckii subsp. *bulgaricus* $\times 10^6$ cfu ml^{-1}	11–680	5–360 <1–150	5–400
Suggested advisory standards:			
	Satisfactory	Doubtful	Unsatisfactory
S. thermophilus $\times 10^6$ cfu ml^{-1}	>100	100–10	<10
L. delbrueckii subsp. *bulgaricus* $\times 10^6$ cfu ml^{-1}	>100	100–10	<10
Coliforms	<1	1–10	>10
Yeasts } cfu ml^{-1}	<10	10–100	>100
Moulds	<1	1–10	>10

After: Davis and McLachlan (1974) and Robinson (1976).

The wide variations are a reflection of both between batch and between brand differences, but the standard suggested by Davis and McLachlan (1974) for a satisfactory yoghurt should be readily attainable. Obviously, it is not suggested that unsatisfactory counts are a cause for concern but, at the same time, it is probably true that if flavour and acid development are satisfactory, then figures of 10×10^6 cfu ml^{-1} species will be an inevitable consequence; results of this order are, therefore, a fair indication that the organoleptic properties of the yoghurt will be satisfactory as well. At the other extreme, a tendency towards extremely high counts may raise problems later, particularly if the refrigeration chain is substandard; consumer complaints relating to excessively sour yoghurts can imply that acid production by *L. delbrueckii* subsp. *bulgaricus* has been poorly controlled.

An examination of yoghurt for contaminant organisms is, as indicated earlier, concerned with protection of the consumer from any potentially pathogenic species and assurance that the material will not undergo microbial spoilage during its anticipated shelf life (Stannard, 1997). These issues are of vital importance to any company. Thus, apart from the moral obligation that a company has to its customers, the financial losses that can accrue from the release of suspect products are motivation enough to give microbial quality control a high priority. A rapid estimation of the total numbers of lactic acid bacteria in yoghurt can be determined using the electric conductivity method (Yoshida *et al.*, 1987).

As far as pathogens are concerned, yoghurt with an acidity of around 1 g 100 g^{-1} lactic acid is a fairly inhospitable medium and really troublesome pathogens like *Salmonella* spp. and *Listeria monocytogenes* will be incapable of growth (Hobbs, 1972). A degree of survival of *L. monocytogenes* at pH 4.5 in labneh has been reported (Gohil *et al.*, 1996) but, even under severe test conditions, the counts

declined rapidly within 24 hours, that is, long before the product would have reached the consumer. Schaack and Marth (1988a–c) observed that *L. monocytogenes* was inhibited during a yoghurt fermentation, but Choi *et al.* (1988), Massa *et al.* (1991), Khattab *et al.* (1993), Zuniga Estrada *et al.* (1995) and Ribeiro and Carminati (1996) suggested that the final pH of the product was important, as was the precise strain of *L. monocytogenes* (see also Siragusa and Johnson, 1988; Ahmed, 1989; Greenwood *et al.*, 1991). However, bacteriological methods for the detection of contaminating micro-organisms and pathogens in milk and milk products have been reported by IDF (1991g, 1994b, 1995d).

Coliforms should also be inactivated by the low pH (Feresu and Nyati, 1990) and, in addition, some species may be susceptible to antibiotics released by the starter organisms (see Chapter 7). The acid sensitivity of *Campylobacter* spp. suggests that contaminants will not survive a normal fermentation (Cuk *et al.*, 1987; Uradzinski, 1990; Ionkova, 1990), but whether *Staphylococcus* spp., and in particular coagulase-positive strains (Masud *et al.*, 1993), can survive in yoghurt is a matter of some dispute (Arnott *et al.*, 1974; Alkanahl and Gasim, 1993). To date there have been no records of staphylococcal food poisoning being associated with the consumption of yoghurt in the United Kingdom (Gilbert and Wieneke, 1973; Keceli and Robinson, 1997) and Attaie *et al.* (1987) showed that a virulent strain of *Staphylococcus aureus* was inhibited during fermentations involving either yoghurt cultures or *L. acidophilus*. For this reason, an examination for staphylococci is not normally required for yoghurt (see Table 10.11) and even the test of fresh yoghurt for coliforms is probably of more value as an indicator of plant hygiene than as a warning that the product may constitute a health risk.

However, this general confidence does have to be tempered with caution, because a recent report linked an outbreak of *Escherichia coli* 0157 with the consumption of yoghurt (Morgan *et al.*, 1993; see also Kornacki and Marth, 1982; Reinheimer *et al.*, 1990; Martin and Marshall, 1995), so that it should be remembered that low starter activity and/or post-heat treatment contamination can lead to problems even with this traditionally safe product (Al-Mashhadi *et al.*, 1987; Ibrahim *et al.*, 1989). This latter point has been emphasised by studies with some of the so-called emerging pathogens like *Yersinia enterocolitica* and *Aeromonas hydrophila*, in which survival in yoghurt or in bio-yoghurt has been shown to be closely correlated with pH (Mantis *et al.*, 1982; Ahmed *et al.*, 1986; El-Kholy, 1992; El-Gmiey *et al.*, 1994; Aytac and Ozbac, 1994a, b; Ozbac and Aytac, 1995, 1996); the behaviour of *Bacillus cereus* in yoghurt will follow a similar pattern (Wong and Chen, 1988; Sultan *et al.*, 1988; Stadhouders and Driessen, 1992). However, many researchers have studied the behaviour of yoghurt starter cultures with antibacterial properties against pathogens, and the following reports are recommended for further reading (Mohran and Said, 1990; Bodana and Rao, 1990; Mohammed and Younis, 1990; Prasad and Ghodeker, 1991; Amin *et al.*, 1991; Bielecka *et al.*, 1994a, b; Balasubramanyam and Varadaraj, 1995; Ebringer *et al.*, 1995; Massa *et al.*, 1997; Yang *et al.*, 1997).

The freak occurrence of *Clostridium botulinum* in hazelnut yoghurt also highlights the need for vigilance (O'Mahony *et al.*, 1990; Collins-Thompson and Wood, 1993), but perhaps of more significance is the finding by Leyer and Johnson (1992) that *Salmonella typhimurium* can display a degree of adaptation to acidity (see also Hosoda *et al.*, 1992; Nadathur *et al.*, 1994). Clearly there is major difference between adaptation to a pH above 5.0 and adaptation to the pH of 4.0–4.2 found in yoghurt,

but with some mild yoghurts being produced at around pH 4.6, the situation needs to kept under observation.

More significant from the producer's standpoint is the examination for yeasts and moulds, for these organisms are capable of spoiling yoghurt well within an anticipated sell-by date. Thus, many fungi are little affected by low pH and with ample sucrose and/or lactose available as energy sources, unacceptable deterioration can be rapid. Yeasts, whether lactose utilisers like *K. marxianus* var *marxianus* and *K. marxianus* var *lactis* or more cosmopolitan species, such as *Saccharomyces cerevisiae* or *Torulopsis candida* (Jordano *et al.*, 1991b) are a major concern (Fleet, 1990a, b; see also Giudici *et al.*, 1996). In order to avoid in-carton fermentation – often manifest by a "doming" of the lid of a carton or collapse of the carton (Anon., 1987b; Foschino *et al.*, 1993), Davis *et al.* (1971) have suggested that yoghurt, at the point of sale, should contain ≤100 viable yeast cells ml^{-1}. Above 1.0×10^3 cfu ml^{-1} would imply a serious risk of deterioration for, although serious gas production and off-flavour development may not be manifest until the yeast population reaches 1.0×10^5 cfu ml^{-1}, such counts can be achieved quite easily within a 2–3 week shelf life (Jordano and Salmeron, 1990; Jordano *et al.*, 1991a). A rapid test method for the detection of one yeast cell per pot in cultured milk products within 24 hours was reported by de Groote *et al.* (1995).

Moulds tend, on the whole, to develop more slowly than the yeasts and although some genera like *Aspergillus* can form button-like colonies within a coagulum, most fungi require oxygen for growth and sporulation. Hence, moulds are usually visible only in retail cartons of set yoghurt, since the surface of stirred yoghurt rarely remains undisturbed for any length of time. Nevertheless, occasional problems can arise from such genera as *Mucor*, *Rhizopus*, *Aspergillus*, *Penicillium* or *Alternaria* and the unsightly superficial growths of mycelium will lead to consumer complaints (Garcia and Fernandez, 1984). For this reason, a mould count of up to 10 cfu ml^{-1} of retail product has been rated as doubtful quality by Davis *et al.* (1971).

It has been reported by Jordano *et al.* (1989) that some strains of *Aspergillus flavus* isolated from commercial yoghurts were aflatoxigenic but, although the sucrose content of fruit yoghurt would be sufficient to support aflatoxin production (Ahmed *et al.*, 1997), it has not been suggested that aflatoxin synthesis does occur in yoghurt. Aflatoxin M_1 has been identified on occasion in the milk for yoghurt production, but even this contamination may, depending on the pH of the product, decline during fermentation (Wiseman and Marth, 1983; Sharaf *et al.*, 1988; El-Deeb, 1989; Batish *et al.*, 1989; Karunaratne *et al.*, 1990; Rasic *et al.*, 1991; Hassanin, 1994; Gourama and Bullerman, 1995a, b; Garcia *et al.*, 1995; El-Nezami and Ahokas, 1998). However, in a recent survey in Italy, the occurrence of aflatoxin M_1 in yoghurt (n = 91) was 80% of the samples, and the amounts ranged between <1 and 497 ng l^{-1}; only two samples had levels that exceeded the Swiss legal limit (>50 ng l^{-1}) (Galvano *et al.*, 1998).

At one time, fruit was the major source of fungal contamination (Fleischer *et al.*, 1984), but now that most sources will be heat treated prior to use, infection from this source should have been eliminated. Airborne spores or yeast cells can prove more difficult to control, particularly during certain months of the year and, unless a serious lapse in plant hygiene is suspected, high yeast or mould counts usually indicate contaminants in the atmosphere. The unexpected variety of yeast species isolated from yoghurt by Tilbury *et al.* (1974) and Suriyarachchi and Fleet (1981) can probably be explained by this type of chance contamination and protection

of the filling area is a top priority. Regular monitoring of the air in the processing area may help to identify the route taken by airborne propagules (Lück and Gavron, 1990) and the examination of representative samples of the end product employing acidified malt agar or Rose Bengal agar (Bridson, 1990), yoghurt whey agar (Yamani, 1993) or chloramphenicol agar (yeasts) (IDF, 1990d) can provide a warning of impending problems. Alternatively, impedance measurements can be employed to determine low levels of yeast in yoghurt (Shapton and Cooper, 1984; Pettipher, 1993; Bolton and Gibson, 1994; Kleiss *et al.*, 1995) and the direct epifluorescent filter technique (DEFT) has been used succesfully by Rowe and McCann (1990) and McCann *et al.* (1991). More recently, the Petrifilm™ method has been recommended by Vlaemynck (1994) for enumerating yeasts and moulds in yoghurt, as has the ISO-GRID membrane filtration system in conjunction with YM-11 agar (Entis and Lerner, 1996; see also Salih *et al.*, 1990).

In commercial practice, any yeast infection is regarded with dismay, and eradication of the source becomes a priority. On occasion, this search may require the identification of the dominant spoilage yeast and the classic texts of Kreger-van Rij (1984) or Barnett *et al.* (1990) can prove valuable sources of information. However, traditional taxonomic methods can be extremely time consuming, and more rapid systems relying on oligonucleotide probes or polymerase chain reaction (PCR) fingerprinting have now been developed (IDF, 1998).

Overall, therefore, it is clear that well-made yoghurt should not present a manufacturer with many complaints as far as microbiological quality is concerned, although some small producers have yet to match the standards of the major suppliers (Tamime *et al.*, 1987).

10.5.3.2 Bio-yoghurts

All the yoghurts in this group, whether labelled as "bio" or "B/A" products, should contain high counts of a "health promoting" culture, such as *L. acidophilus*, *Bifidobacterium* spp. or similar organisms, where high means above the therapeutic minimum discussed earlier. However, few commercial products contain just *L. acidophilus* and/or *Bifidobacterium*, so that any system for quality control must be able to cope with the presence of *S. thermophilus* and perhaps *L. delbrueckii* subsp. *bulgaricus* as well. Consequently, a range of media has evolved for examining bio-yoghurts for the presence of *L. acidophilus* alone in a fermented milk, or in the presence of other genera, and a selection of these media are shown in Table 10.12 (see also Lim *et al.*, 1995; Lankaputhra *et al.*, 1996; Dave and Shah, 1997; Micanel *et al.*, 1997).

The choice of medium does, as mentioned elsewhere, depend on the personal preference of the operator, whether the medium needs to be selective for automatic counting, or whether differentiation between a number of species on one plate is an advantage and the strains of the species concerned, because the reactions in any medium are usually strain dependent. Some media, such as TPPYPB, are good for differentiating the normal yoghurt microflora, but offer the additional advantage of enabling an inoculum of a yoghurt culture, bifidobacteria and *L. acidophilus* to be enumerated on one medium (Ghoddusi and Robinson, 1996). Thus, colonies of *S. thermophilus* appear as small pale blue colonies with a thin blue zone in RCPB agar, while *Bifidobacterium* spp. give rise to white colonies. *L. delbrueckii* subsp. *bulgaricus* produce small, shiny white colonies surrounded by a wide, royal blue zone and *L. acidophilus* are readily distinguished as large, pale blue colonies surrounded

Table 10.12 Some of the media which can be employed to enumerate *L. acidophilus* and other "health-promoting" lactobacilli when growing either alone in milk, or in the presence of other lactic acid bacteria

Culture medium	Species	Comments
L. acidophilus alone		
MRS agar	*L. acidophilus*	Non-selective
Tomato juice agar	*L. acidophilus*	Non-selective
L. acidophilus in the presence of *Leuconostoc* or *Lactococcus* spp.		
Aesculin-cellobiose agar	*L. acidophilus*	The ability of *L. acidophilus* to hydrolyse aesculin and ferment cellobiose at 40°C makes this medium selective.
X-Glu-agar	*L. acidophilus*	Mesophiles lack the β-D-glucosidase enzyme which is the basis for the colour reaction produced by *L. acidophilus*.
L. acidophilus in the presence of thermophilic lactic acid bacteria		
MRS-bile agar	*L. acidophilus*	Bile salts inhibit yoghurt cultures and aerobic incubation restricts the growth of bifidobacteria.
TGV agar	Non-selective	Colonies distinguished on basis of colour and morphology.
LA agar	Non-selective	Colonies distinguished on basis of colour and morphology.
TPPYPB agar	Non-selective	Colonies distinguished on basis of colour and morphology.

Note: The media may have to be modified for counting of *L. reuteri* or *L. rhamnosus*; some species of bifidobacteria can utilise cellobiose.

After: IDF (1995a) and Ghoddusi and Robinson (1996).

by a wide, royal blue zone; Rybka and Kailasapathy (1996) proposed a similar scheme employing RCPB agar. It must be emphasised again that differences between strains may prove to be important and it may be that a number of the media mentioned in Tables 10.5 to 10.7 are capable of further modification and improvement.

If *Bifidobacterium* spp. alone are the organisms of interest, then species of human origin grow well on a number of media (see Table 10.13). However, in the presence of other lactic acid bacteria, selective agents have to be employed and the choice and level of the agents can have a dramatic impact on recovery. Thus as shown in Table 10.14, on blood liver agar supplemented with neomycin sulphate, paramomycin sulphate, nalidixic acid and lithium chloride (NPNL), only *Bifidobacterium bifidum* showed acceptable growth. The same contrast in growth responses was observed on modified rogosa agar with NPNL added at 50 ml l^{-1} of medium, and also with TPY agar. Successful growth of both species of *Bifidobacterium* was recorded in the presence of lithium chloride/sodium propionate, but unfortunately both mesophilic and other thermophilic lactic acid bacteria grow on the same medium. The most promising option, therefore, appears to be the use of TPY agar with 20 ml l^{-1} NPNL which gives good recovery for both *B. bifidum* and *Bifidobacterium adolescentis* while, at the same time, eliminating the growth of other cultures.

Table 10.13 Some of the media which can be employed to enumerate *Bifidobacterium* spp. growing either alone in milk or in the presence of other lactic acid bacteria under anaerobic conditions at 37°C

Culture medium (agar)	Selective supplement	Response of *Bifidobacterium* sp.
Blood liver	Nil	Excellent growth
Modified rogosa	Nil	Excellent growth
de Man rogosa sharpe	Nil	Limited growth
Tomato juice	Nil	Excellent growth
Trypticase phytone yeast	Nil	Excellent growth
Lithium chloride/Na-propionate	Nil	Excellent growth[a]
AMC[f]	–	Specified for *Bifdobacterium longum*
Blood liver	NPNL[c]	Good growth/selectivity[b]
Modified rogosa	NPNL	Good growth/selectivity
Modified rogosa	PPNL[d]	Excellent growth/selectivity
Trypticase phytone yeast	NNL[e]	Excellent growth/selectivity

[a] This medium may NOT be selective against certain strains of cheese or yoghurt starter cultures. [b] Recovery of *Bifidobacterium* spp. depends on the species, strain of the species and the concentration of NPNL. [c] NPNL (mg 100 ml^{-1} of stock solution): neomycin sulphate (10), paramomycin sulphate (20), nalidixic acid (1.5), lithium chloride (300) with an addition rate of 2–5 ml of stock solution 100 ml^{-1} of medium. [d] PPNL (mg 100 ml^{-1} of stock solution): sodium propionate (6000), paramomycin sulphate (200), neomycin sulphate (800), lithium chloride (12 000) with an addition rate of 5 ml 100 ml^{-1} of medium. [e] NNL (mg 100 ml^{-1} of stock solution): neomycin sulphate (200), nalidixic acid (30), lithium chloride (6000) with an addition rate of 5 ml 100 ml^{-1} of medium. [f] AMC from work by Arroyo *et al.* (1995).

After de Man *et al.* (1960), Shimada *et al.* (1977), Teraguchi *et al.* (1978), Scardovi (1986), Rasic (1990), Samona and Robinson (1991), Lapierre *et al.* (1992), Arroyo *et al.* (1995) and Tamime *et al.* (1995).

Table 10.14 Total colony counts ($\times 10^6$ cfu ml^{-1}) of the organisms indicated on a range of general and selective media that have been recommended for the enumeration of bifidobacteria

Medium (agar)	Supplement	*B. bifidum*	*B. adolescentis*
Blood liver	–	510	850
	NPNL[a]	280	0
	PPNL[a]	0	0
Modified rogosa	–	202	720
	NPNL	180	0
	PPNL	0	0
TPY	–	250	790
	NPNL	340	0
	PPNL	0	0
	NPNL[b]	190	680
	LP[c]	630	1140

[a] The compositions of these supplements are shown in Table 10.13. [b] The NPNL concentration is 2 ml 100 ml^{-1}. [c] LP (g 100 ml^{-1}): lithium chloride (0.2) and sodium propionate (0.3); each composite solution was added to the basic medium at a rate of 5 ml 100 ml^{-1}; this supplement is NOT selective against all strains of yoghurt or cheese cultures.

Note: The plates were incubated anaerobically at 37°C.

After Ghoddusi (1996).

However, the need to check any proposed medium against the strains being employed in the factory is emphasised by the fact that, while *Lactococcus* species (NCDO 276) grew on TPY agar with 20 ml^{-1} NPNL, *Lactococcus* species (NCDO 763) was inhibited; similar patterns could probably observed with strains of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* as well. Unless colony morphology/colour eliminates confusion, it is important to note that checks on typical colonies under the microscope may not prove helpful. Thus, while the cells of bifidobacteria are usually Y-shaped or bone-shaped depending upon the species (Tamime *et al.*, 1995), they may become coccoid if the growing conditions so dictate (Samona and Robinson, 1991). Consequently, the observation of Gram-positive cocci in a breed smear of a colony from a plate of a medium presumed to be selective could result from the presence of either normal *S. thermophilus* or abnormal *Bifidobacterium* sp.; cell shape and size would provide little help. It may be worth mentioning, however, that the change in morphology tends to occur over several generations, so that the examination of a bio-yoghurt manufactured with a direct-to-vat culture should reveal typical Y-shaped or bone-shaped cells.

In addition, it should be emphasised that the concentration of any antibiotic mixture can have an impact on the total level of recovery of bifidobacteria (Samona and Robinson, 1991) and, bearing in mind that health claims should be supported by high cell counts in the product, media selection becomes a vital issue.

10.5.4 Assessment of organoleptic characteristics

The ultimate judge of any product in a free society is the consumer and although brand awareness only accounts for some 20% of decisions of purchase (Kroger and Fram, 1975), deliberate avoidance of a brand as the result of dissatisfaction represents a completely separate situation. To some extent, the chemical and physical analyses suggested earlier (e.g. titratable acidity and viscosity) will provide a reasonable indication that the normal in-house standards have been achieved, but the use of some form of taste panel to perform a final check is usual practice. The composition of such panels can range from "one man and a plastic teaspoon" through to a full panel of trained tasters selected and organised along the lines proposed by Amerine *et al.* (1965), Stone and Sidel (1985) and Lyon *et al.* (1992). Obviously no one would dispute the skill of the individual (Harper, 1962, 1972, 1977), but the more objective and quantifiable the acquired data can be made, the easier the task of maintaining standards over a long period of time.

This latter approach is, of course, time-consuming and may involve:

- convening a panel of at least five judges (with alternatives) on the basis of their knowledge of the product and their willingness to participate on a regular basis;
- obtaining agreement among the judges on the characteristics of a good quality yoghurt, with the definition of good quality being solely related either to products from the factory in question or to a specific brand image;
- obtaining agreement among the judges about what is meant by the terminology that might be applied to certain faults or defects; and
- the derivation of a scheme of assessment that can be employed as part of a routine quality control procedure.

The ultimate selection of a scheme will rest with the panel concerned, but three typical schemes that have been proposed and/or employed in various countries are shown in Tables 10.15, 10.16 and 10.17 (Fütschik, 1963; Bergel, 1971a; Pearce and

Heap, 1974; Bodyfelt *et al*., 1988; see also Grab, 1983). The over-riding factors must be operational simplicity and the ability of the procedure to discriminate between samples.

A few practice runs will quickly establish the preferences of a particular panel and it should then be possible to accept, perhaps with modification, one of the available schemes. It is worth noting, however, that the description of defects can be a valuable part of the exercise, because the quality controller may then be in a position to indicate why the particular batch of yoghurt has scored poorly in certain respects (see also Bodyfelt *et al*., 1988; Ogden, 1993; IDF, 1997d). Thus, as shown in Table 10.18, some degree of association between a recognised faults and likely causes does exist, and hence an accurate description from a taste panel can speed up the implementation of remedial action.

In some instances the causes of defects are not readily identifiable, the apparently seasonal occurrence of granulation in yoghurt is a case in point (Robinson, 1981). Thus, although there is evidence linking poor process control with the formation of small, protein-rich lumps in yoghurt, a fault especially noticeable in fruit yoghurts, there have been reports that the defect is most prominent during the spring and autumn months (Cooper *et al.*, 1974). Whether this periodicity is linked with seasonal changes in milk composition has not been established, nor is it clear

Table 10.15 Yoghurt Evaluation – Scheme I – The Karl Ruher Nine Point Scheme

Score	Judgement	Quality	Range	Class	Overall classification
9	Excellent	–		Upper	
8	Very good	Very good	I	Medium	Free of objections
7	Good	Good		Lower	
6	Satisfactory	Satisfactory		Upper	
5	Mediocre	Average	II	Medium	Still acceptable
4	Sufficient	Sufficient		Lower	in commerce
3	Imperfect	Bad		Upper	
2	Bad	Bad	III	Medium	Unsaleable
1	Very bad	Bad		Lower	

Table 10.16 Yoghurt Evaluation – Scheme II

Date:
Taster: Code No:

a.	Appearance and colour	.....
	Defects	.....
b.	Body and Texture	.....
	Defects	.....
c.	Flavour	.....
	Defects	.....
	Overall score	.....

Judge the three characteristics on a 1–5 scale of: 5 excellent; 4 very good; 3 good; 2 fair; 1 poor.

The overall score is obtained by multiplying the flavour score by 2 and then adding that score to the rest. An excellent yoghurt gives an overall score of 20.

Possible defects: (a) appearance and colour (extraneous matter, lack of uniformity, unnatural colour, surface discoloration, wheying-off, fat separation, gassiness), (b) body and texture (too thin, gelatinous, chalky, lumpy or granular, slimy), and (c) flavour (excess acid, excess sugar, excess stabiliser, excess milk powder, yeasty, unclean).

Table 10.17 Yoghurt Evaluation – Scheme III – Approved by the American Dairy Science Association

Yoghurt Score Card	
Attribute	Perfect score
Flavour	10
Body and Texture	5
Appearance	5
Total	20

Defects: (a) flavour (acetaldehyde – coarse, bitter, cooked, foreign, high and/or acid, lacks flavour; flavouring – freshness and/or sweetness, old ingredient, oxidised, rancid, too high flavouring and/or sweetness, unnatural flavour, unclean), (b) body and texture (gel-like, grainy, ropy, too firm, weak), and (c) appearance (atypical colour, colour leaching, excess fruit, free whey, lacks fruit, lumpy, shrunken, surface growth).

why some manufacturers observe the problem more than others, why changes in starter culture can often solve the problem, and why reversion to the original culture after 2–3 weeks does not lead to reoccurrence of the problem; applying a high shear to yoghurt after manufacture reduced nodulation, but also reduced viscosity (Guirguis *et al*., 1987). Furthermore, it may be likely that during the fermentation stage, the starter organisms clump and generate a region of low pH causing isoelectric precipitation of casein in and around the clumps (Weeks *et al*., 1997).

Schemes of the type cited earlier have the further attraction of being easy to operate once the panel has become familiar with the product and the use of the form but, at the end of the day, they do remain essentially subjective. For this reason, there has been much attention paid to the possibility of imposing a more rigid framework within which the taste panel might operate. To this end, Robinson (1988) applied qualititative descriptive analysis (QDA) as described by Powers (1988) to natural yoghurts produced with different starter cultures. Ten terms were employed to describe the flavour or mouthfeel of the yoghurts, and the attribute profiles were easily distinguished (see Fig. 10.11). The scheme is equally applicable to stirred fruit/flavoured yoghurts and, once the terms covering a typical retail sample are agreed by a panel and the profile drawn, the profiles for subsequent samples can be compared by superimposition. By performing this operation on a weekly basis, changes in perceived quality can be readily detected; experience will soon indicate whether an observed difference between the sample and the standard profiles is significant.

Multidimensional scaling procedures (i.e. KYST and SINDSCAL) have been used by Poste and Patterson (1988) to identify yoghurt characteristics by trained panelists. Nine selected attributes were selected by the panellists, and the presence of fruit, sweetness, acidity and lumpiness of yoghurt appeared to be predominant in the perceived interrelationships. However, Tuorila *et al.* (1993) reported the sensory results of a trained panel ($n = 14$) and consumer panel ($n = 41$), and while perceived sweetness and creaminess were interrelated, acid taste was not; yoghurt samples with sucrose $10\,g\,100\,g^{-1}$ and fat $3.5\,g\,100\,g^{-1}$ received the highest scores, whilst more men than women preferred sweet and creamy yoghurts.

In an attempt to determine which organoleptic attributes were important with respect to consumer perceptions of quality, Muir and Hunter (1992) asked a panel

Table 10.18 Some common defects of yoghurt and an indication of some possible causes and remedies

Defect	Possible causes	Remedy
Syneresis	Low SNF or fat content	Adjust formulation
	High mineral content in milk	Blend with milk of low mineral content
	Insufficient heat treatment/ homogenisation of milk	Adjust process conditions
	Too high incubation temperature	Reduce temperature to 42°C
	Low acidity, e.g. pH 4.8	Ensure pH 4.4
	Adventitious enzymes capable of coagulating protein	Eliminate source
	Disturbance of coagulum prior to cooling	Adequate cooling
	Unspecified	Addition of stabiliser Change to culture of viscous type
Low viscosity	Low total solids	Adjust formulation
	Insufficient heat treatment/ homogenisation of milk	Adjust process conditions
	Too low incubation temperature	Raise temperature to 42°C
	Too low inoculation rate	Raise inoculum to 2 ml 100 ml^{-1} or equivalent
	Prolonged agitation	Improve mechanical handling system
	Unspecified	Addition of stabiliser Change culture to viscous type
Bubbles in coagulum (in gel)	Poor storage conditions	Check temperature of cool stores
	Contamination with yeasts	Eliminate source of infection
	Contamination with coliforms	Poor plant or starter hygiene
	Excessive aeration of mix	Control agitation
Granular coagulum	Poor mixing of milk powder	Adjust process conditions
	Agitation prior to cooling	Adequate cooling
	Too high incubation temperature	Reduce temperature to 42°C
	Too low inoculation rate	Raise inoculum to 2 ml 100 ml^{-1} or equivalent
	Unspecified	Change culture to viscous type
Flavour problems	Insipid	Lower inoculum to 2 ml 100 ml^{-1} or equivalent Extend incubation time
	Unclean	Raise inoculum to 2 ml 100 ml^{-1} or equivalent Reduce incubation time Check for contamination with coliforms
	Bitter	Lower inoculum to 2 ml 100 ml^{-1} or equivalent Change starter culture
	Sour	Lower inoculation rate Check temperature to storage
	Malty/yeasty	Suspect contamination by yeasts
	Rancid	Check quality of raw milk

See also Connolly (1990).

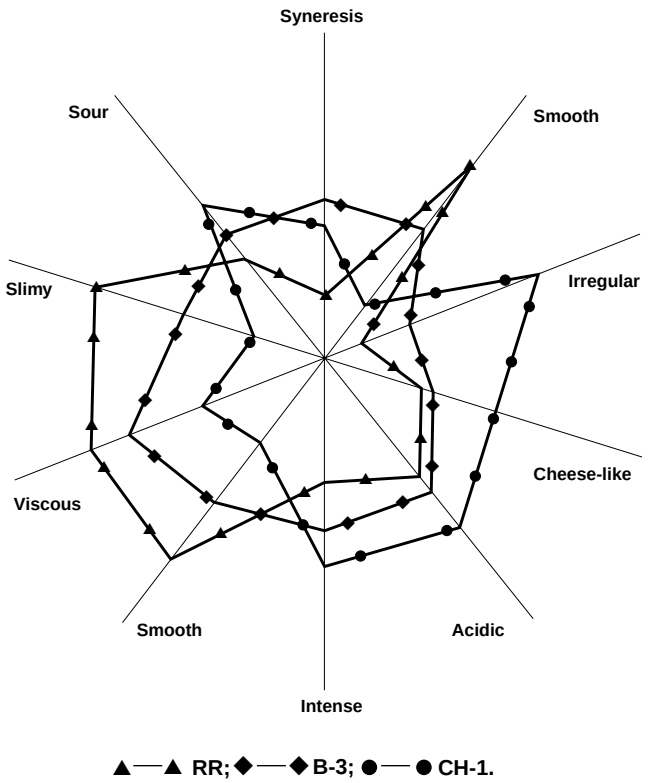


Fig. 10.11 Attribute profiles of natural yoghurts made with three different cultures

Culture RR was obtained from NIZO in the Netherlands and cultures B-3 and CH-1 from Chr. Hansen's Laboratory in the UK.

Reproduced by courtesy of *Dairy Industries International*.

of 20 judges familiar with fermented milks to examine nine different types of commercial natural yoghurt/yoghurt-style product, and suggest terms that described the sensory properties of one or more of the samples. The samples ranged from low fat fromage frais through to Greek-style yoghurt with 10 g fat 100 g^{-1} and the consensus was that the following terms were important:

Odour	Flavour	Aftertaste	Texture
Intensity	Intensity	Intensity	Firmness
Sour	Sour/acid	Bitter	Creaminess
Fruity	Fruity	Sour/acid	Viscosity
Buttery	Buttery	Other	Sliminess
Yeasty	Rancid		Curdy
Creamy	Creamy		Mouth-coating
Sweet	Salty		Chalky
Other	Bitter		Serum separation
	Lemon		
	Sweet		
	Chemical		
	Other		

The application of QDA under rigorously controlled conditions (MacFie *et al.*, 1989) provided data for analysis by principal component analysis (Piggott, 1988) and the results of Muir and Hunter (1992) revealed a number of important points with respect to the sensory analysis of yoghurt, namely:

- giving the panel the option of the term "other" generates a plethora of descriptors that are not helpful;
- the data could be simplified into five principal components responsible for over 90% of the total variance, i.e.

Odour:	Flavour:	After-taste:	Texture:
Sweet	Intensity	Acid	Firmness
	Acid/sour		Creamy
	Fruity		Viscosity
	Creamy		Curdy
	Lemon		Chalky
	Sweet		Serum separation

In other words, it would appear that five attributes are important in discriminating between and/or describing fermented milks: acidity, curd character, sweetness, creamy character and chalkiness. However, the further application of this conclusion would merit caution, because consumer reaction to acidity, for example, can be positive or negative depending on the market (Greig and van Kan, 1984). The reaction to sweetness can again vary with the market and the growing demand for mild flavoured, sweet yoghurts in the U.K. suggests that a large sector of the public link sucrose content with acceptability (McGregor and White, 1986). Evidence from the retail sector would support the view that a creamy mouthfeel is strongly correlated with a perception of quality and many stirred fruit yoghurts now contain over $1.0\,g\,100\,g^{-1}$ milk fat as routine. The isolation of curd character and chalkiness is probably a reflection of the restriction of the procedure to natural yoghurts, often of the set variety, and the wider usefulness of these terms might merit further study.

It is of note also that none of the panellists appears to have suggested the term "yoghurt-like", because the flavour of natural yoghurt, based upon acetaldehyde and similar components, is quite unique among the fermented milks (Hruskar *et al.*, 1995). Thus, the use of a generic name to describe the flavour of a product has much to recommend it (Harper, 1962) and it would be interesting to know what panellists were actually describing as intensity. Nevertheless, this more objective approach to sensory analysis will help to define those characters of a product that are important with respect to acceptability, so enabling manufacturers to refine their own routine assessments.

To this end, some additional sensory studies on yoghurt have included first, preference mapping that allows the investigator to relate the preference responses of consumers to a map where the results can be related to product formulations (Anon., 1998b). Gains and Gutteridge (1991) evaluated different British yoghurts using this technique and they reported that the rank order of the yoghurts preferred by consumers were population 1 – thick and creamy, population 2 – natural, and population 3 – low fat. Second, using different types of starter cultures to make yoghurt, Rohm *et al.* (1994) observed that the trained assessors could easily identify differences in each sensory categories of these products except gel firmness using a hedonic scale. Multiple regression analysis revealed that the results were mainly determined by flavour and EPS cultures, showing positive and negative

weightings, respectively. Third, Stoer and Lawless (1993) concluded from their organoleptic study (n = 920) of dairy products including yoghurt that both single product scaling and relative-to-product scaling methods of assessment by trained and untrained panellists were equal in their efficiency for sensory evaluation. Fourth, detailed studies of the sensory ratings of commercial yoghurts (plain and fruit flavoured) by a consumer panel (n = up to 180) and a descriptive panel (n = 11) have been reported by Barnes *et al*. (1991a, b) and Harper *et al*. (1991) (see also Muir *et al*., 1997; Kähkönen *et al*., 1997). Fifth, taste and health claims for yoghurt had the largest influence on buying intent of American consumers, whilst brand had little influence (Vickers, 1993).

10.6 Conclusions

If the essential requirements for manufacturing a high quality yoghurt were to be tabulated (see also Lewis and Dale, 1994), then it is likely that the list might look rather like this:

- milk of good quality and adequate SNF;
- correct heat treatment;
- an active, well-balanced and contaminant-free starter culture;
- a clean and well-maintained plant;
- correct inoculation rate;
- correct incubation times and temperatures;
- avoidance of rough handling of set yoghurts;
- the use of high quality fruit or other ingredients;
- correct storage of retail product below 5°C,

and what is important about this list is that all these areas should form part of the commitment to good manufacturing practice. The actual degree of surveillance will vary in the light of experience in a particular plant, but the principle remains the same, namely that someone in authority must have an accurate picture of the entire operation, for without this, the smooth running of the plant and the quality of the end product will be at risk.

10.7 References

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